PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classificati n 6 :
C07K 7/06, 7/08, 7/10, A61K 38/00

A1

(11) Internati nal Publication Number: WO 97/12905

(43) Internati nal Publication Date: 10 April 1997 (10.04.97)

(21) International Application Number: PCT/US95/12968

(22) International Filing Date: 6 October 1995 (06.10.95)

(60) Parent Applications or Grants
(63) Related by Continuation
US 08/042,318 (CIP)
Filed on 2 April 1993 (02.04.93)
US 08/142,590 (CIP)
Filed on 25 October 1993 (25.10.93)
US PCT/US94/03520 (CIP)
Filed on 30 March 1994 (30.03.94)

(71) Applicants *(for all designated States except US)*: THE GENERAL HOSPITAL CORPORATION [US/US]; 55 Fruit Street, Boston, MA 02155 (US). SHISEIDO CO., LTD [JP/JP]; 7-5-5, Ginza, Chuo-ku, Tokyo 104-10 (JP).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: HIBINO, Toshihiko [JP/US]; CBRC, Building 149, 13th Street, Charlestown, MA 02129 (US). TAKAHASHI, Tadahito [JP/US]; CBRC, Building 149, 13th Street, Charlestown, MA 02129 (US). HORII, Izumi [JP/US]; CBRC, Building 149, 13th Street, Charlestown, MA 02129 (US). GOETINCK, Paul, F. [US/US]; Apartment 2, 282 Marlborough Street, Boston, MA 02116 (US). BACIU, Peter, C. [US/US]; 572 Haverhill Street, Rowley, MA 01969 (US).

(74) Agents: MYERS, Paul, Louis et al.; Lahive & Cockfield, 60 State Street, Boston, MA 02109 (US).

(81) Designated States: AU, CA, JP, KR, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: UROKINASE PLASMINOGEN ACTIVATOR FRAGMENTS

(57) Abstract

Purified uPA peptides containing as few as six amino acids of the EGF-like domain of uPA having mitogenic activity. uPA peptides having a residue other than lysine at position 23.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	**GB**	United Kingdom	**MW**	Malawi
AT	Austria	**GE**	Georgia	**MX**	Mexico
AU	Australia	**GN**	Guinea	**NE**	Niger
BB	Barbados	**GR**	Greece	**NL**	Netherlands
BE	Belgium	**HU**	Hungary	**NO**	Norway
BF	Burkina Faso	**IE**	Ireland	**NZ**	New Zealand
BG	Bulgaria	**IT**	Italy	**PL**	Poland
BJ	Benin	**JP**	Japan	**PT**	Portugal
BR	Brazil	**KE**	Kenya	**RO**	Romania
BY	Belarus	**KG**	Kyrgystan	**RU**	Russian Federation
CA	Canada	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CF	Central African Republic			**SE**	Sweden
CG	Congo	**KR**	Republic of Korea	**SG**	Singapore
CH	Switzerland	**KZ**	Kazakhstan	**SI**	Slovenia
CI	Côte d'Ivoire	**LI**	Liechtenstein	**SK**	Slovakia
CM	Cameroon	**LK**	Sri Lanka	**SN**	Senegal
CN	China	**LR**	Liberia	**SZ**	Swaziland
CS	Czechoslovakia	**LT**	Lithuania	**TD**	Chad
CZ	Czech Republic	**LU**	Luxembourg	**TG**	Togo
DE	Germany	**LV**	Latvia	**TJ**	Tajikistan
DK	Denmark	**MC**	Monaco	**TT**	Trinidad and Tobago
EE	Estonia	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**UG**	Uganda
FI	Finland	**ML**	Mali	**US**	United States of America
FR	France	**MN**	Mongolia	**UZ**	Uzbekistan
GA	Gabon	**MR**	Mauritania	**VN**	Viet Nam

UROKINASE PLASMINOGEN ACTIVATOR FRAGMENTS

This application is a continuation-in-part of USSN 08/142,590, filed on October 25, 1993; PCT/US94/03520 filed March 30, 1994; and of USSN 08/042,318, filed on April 2, 1993; all of which are hereby incorporated by reference.

Background of the Invention

The invention relates generally to urokinase plasminogen activator and fragments thereof.

Two types of plasminogen activators, tissue type plasminogen activator (tPA) and urokinase, or urokinase plasminogen activator (uPA), are known. tPA binds directly to fibrin clots where it activates the conversion of plasminogen to plasmin. uPA is a serine protease with systemic activity. It binds to the receptor found on many cell types and converts plasminogen to plasmin on the cell surface.

Summary of the Invention

uPA is an approximately 55 Kd molecule which consists of (beginning at the N-terminal end) an EGF-like domain (EGF) (which corresponds to residues 1 - 45) a kringle domain (which corresponds to residues 46 - 157), and a trypsin-like protease domain (which corresponds to residues 158 - 411). The EGF and kringle domains make up the amino terminal fragment (ATF), which is mitogenic for human keratinocytes. uPA binds, by its EGF-like domain, to a specific membrane receptor (uPAR) expressed in many cell types. The EGF-like domain is often referred to as the growth factor domain (GFD). The amino acid residue numbering system used herein begins with residue 1 at the N terminal end of uPA.

The inventors have discovered that peptides containing as few as six amino acids of the EGF-like domain of uPA can modulate cell growth. While not wishing to be bound by theory, it is believed that these peptides modulate growth indirectly, by modulating uPA catalytic activity. uPA activity is taught to be modulated by autolysis of uPA to an ATF domain and a proteinase domain. The released ATF fragment is believed to modulate growth by binding to a cell surface heparan sulfate proteoglycan, e.g., a syndecan, e.g., a syndecan-1, 2, 3 or 4, or any combination thereof.

In general, the invention features, a uPA peptide, preferably a purified peptide, which consists essentially of or contains more than 5 and less than 15 (or more preferably more than 5 and less than 19), contiguous amino acid residues from the growth factor domain, e.g., residues 16 through 33, (or more preferably 14 through 33), of urokinase plasminogen activator.

In preferred embodiments, the uPA peptide is capable of either or both binding to or modulating, e.g., inducing or inhibiting, mitogenesis in cells bearing the uPAR and/or cell surface heparan sulfate proteoglycans, e.g., syndecans, e.g., a syndecan-1, 2, 3 or 4, or any combination thereof, e.g., epidermal cells, e.g., keratinocytes. In another preferred

embodiment, the uPA peptide is capable of modulating, e.g., enhancing or inhibiting, uPA catalytic activity.

In preferred embodiments the peptide contains more than 5 and less than 13, (or more preferably more than 5 and less than 16), contiguous amino acid residues, inclusive, of the growth factor domain; the peptide includes the amino acid sequence Thr-Cys-Val-Ser-Asn-Lys-Tyr-Phe-Ser-Asp-Ile-His (SEQ ID NO:6); the peptide contains more than 5 and less than 10 contiguous amino acid residues, inclusive, of the domain growth factor; the peptide includes the amino acid sequence Ser-Asn-Lys-Tyr-Phe-Ser-Asp-Ile-His (SEQ ID NO:1); the peptide contains 6 contiguous amino acid residues of the domain growth factor; the peptide includes the amino acid sequence Ser-Asn-Lys-Tyr-Phe-Ser (SEQ ID NO:2).

In preferred embodiments the peptide is mitogenic for cells bearing the uPAR; the peptide binds to the uPAR but is not mitogenic for cells bearing the uPAR; the peptide is mitogenic for cells bearing cell surface heparan sulfate proteoglycans, e.g., syndecans, e.g., a syndecan-1, 2, 3 or 4, or any combination thereof.

In preferred embodiments, the uPA peptide modulates the growth or mitogenesis of a proliferating cell to a greater extent than it does a cell of the same type which is proliferating at a lesser rate or is not proliferating.

In another aspect, the invention includes a uPA peptide, preferably a purified peptide, which consists essentially of or contains more than 5 and less than 12 contiguous amino acid residues from the amino terminal fragment, e.g., from the growth factor domain, e.g., from residues 15 through 30 of uPA.

In preferred embodiments, the uPA peptide is capable of either or both binding to or modulating, e.g., inducing or inhibiting, mitogenesis in cells bearing the uPAR and/or cell surface heparan sulfate proteoglycans, e.g., syndecans, e.g., a syndecan-1, 2, 3 or 4, or any combination thereof, e.g., epidermal cells, e.g., keratinocytes. In another preferred embodiment, the uPA peptide is capable of modulating, e.g., enhancing or inhibiting, uPA catalytic activity.

In preferred embodiments the peptide contains more than 5 and less than 10 contiguous amino acid residues, inclusive, of the amino terminal fragment of urokinase plasminogen activator; the peptide includes the amino acid sequence Ser-Asn-Lys-Tyr-Phe-Ser-Asp-Ile-His (SEQ ID NO:1); the peptide contains 6 contiguous amino acid residues, inclusive, of the amino terminal fragment of urokinase plasminogen activator; the peptide includes the amino acid sequence Ser-Asn-Lys-Tyr-Phe-Ser (SEQ ID NO:2).

In preferred embodiments the peptide is mitogenic for cells bearing the uPAR; the peptide binds to the uPAR but is not mitogenic for cells bearing the uPAR; the peptide is mitogenic for cells bearing cell surface heparan sulfate proteoglycans, e.g., syndecans, e.g., a syndecan-1, 2, 3 or 4, or any combination thereof.

In preferred embodiments, the uPA peptide modulates the growth or mitogenesis of a proliferating cell to a greater extent than it does a cell of the same type which is proliferating at a lesser rate or is not proliferating.

In another aspect the invention includes the peptide, preferably in a purified preparation, Asn-Gly-Gly-Thr-Cys-Val-Ser-Asn-Lys-Tyr-Phe-Ser-Asn-Ile-His-Trp-Cys-Asn (SEQ ID NO:4).

In another aspect, the invention features a uPA, preferably a purified peptide in which the amino acid residue at position 23 is an amino acid other than L-lysine and: the peptide has a wild type activity; the peptide has less than a wild type activity; the peptide has more than wild type activity.

In preferred embodiments, the uPA peptide has wild type activity if it has one or more of the following: 1) it is capable of either or both of binding to or modulating, e.g., inducing or inhibiting, mitogenesis in cells bearing the uPAR and/or cell surface heparan sulfate proteoglycans, e.g., syndecans, e.g., a syndecan-1, 2, 3 or 4, or any combination thereof, e.g., epidermal cells, e.g., keratinocytes; 2) it is capable of modulating, e.g., enhancing or inhibiting, uPA catalytic activity.

In another aspect, the invention features a uPA, preferably a purified peptide, peptide in which the amino acid residue at position 23 is an amino acid other than L-lysine. In preferred embodiments: the amino acid residue at position 23 is an amino acid having a side chain with a neutral charge at a physiological pH, e.g., at pH 7; amino acid residue at position 23 is an amino acid having a side chain which is neutral as compared to the side chain of lysine; the amino acid residue at position 23 is a neutral amino acid; the amino acid residue at position 23 is any of alanine, glycine, cysteine, or a neutral or neutral non-naturally occurring amino acid.

In another aspect, the invention features a uPA, preferably a purified peptide, peptide in which the amino acid residue at position 23 is an amino acid other than L-lysine. In preferred embodiments: the amino acid residue at position 23 is an amino acid having a side chain with a net-positive charge at a physiological pH, e.g., at pH 7; amino acid residue at position 23 is an amino acid having a side chain which is more positively charged than is the side chain of lysine; the amino acid residue at position 23 is a basic amino acid; the amino acid residue at position 23 is any of arginine, histidine, or a positively charged or basic non-naturally occurring amino acid.

In other preferred embodiments: the amino acid residue at position 23 is an amino acid having a side chain with a net-negative charge at a physiological pH, e.g., at pH 7; the amino acid residue at position 23 is an amino acid having a side chain which is more negatively charged than is the side chain of lysine; the amino acid residue at position 23 is an acidic amino acid; the amino acid residue at position 23 is any of aspartic acid, glutamic acid, or a negatively charged or acidic non-naturally occurring amino acid.

In preferred embodiments the peptide is a full length uPA peptide.

In another aspect, the invention features a uPA peptide, preferably a purified peptide, which includes a sequence of the formula: n-R^2-Asn-R^1-Tyr-Phe-R^3-c, wherein,

R^1 is an amino acid residue other than L-lysine;

R^2 is a sequence of between 1 and 21 residues in length, having as its carboxy-terminus Ser^{21} of uPA and extending, inclusive of Ser^{21}, from between 1 and 21 amino acid residues in the N-terminal direction of uPA; and

R^3 is a sequence of between 1 and 25 residues in length having as its amino-terminus Ser^{26} of uPA and extending, inclusive of Ser^{26}, from between 1 and 25 amino acid residues in the C-terminal direction of uPA;

wherein c indicates the carboxy terminal direction of the peptide and n indicates the amino terminal direction of the peptide.

In preferred embodiments: R^1 is an amino acid having a side chain with a neutral charge at a physiological pH, e.g., at pH 7; R^1 is a neutral amino acid; R^1 is any of alanine, glycine, cysteine, or a neutral or neutral non-naturally occurring amino acid.

In preferred embodiments: R^2 is any of:

n-Asp-Cys-Leu-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:9);
n-Cys-Leu-Asn-Gly-Gly-Thr-Cys-Val-Ser-c; (SEQ ID NO:10)
n-Leu-Asn-Gly-Gly-Thr-Cys-Val-Ser-c; (SEQ ID NO:11)
n-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:12);
n-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:13);
n-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:14);
n-Thr-Cys-Val-Ser-c (SEQ ID NO:15);
n-Cys-Val-Ser-c;
n-Val-Ser-c; or
n-Ser-c

In preferred embodiments: R^3 is any of:

n-Ser-c;
n-Ser-Asn-c;
n-Ser-Asn-Ile-c;
n-Ser-Asn-Ile-His-c (SEQ ID NO:16);
n-Ser-Asn-Ile-His-Trp-c (SEQ ID NO:17);
n-Ser-Asn-Ile-His-Trp-Cys-c (SEQ ID NO:18); or
n-Ser-Asn-Ile-His-Trp-Cys-Asn-c (SEQ ID NO:19).

In preferred embodiments: R^2 is n-Asp-Cys-Leu-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:9) and R^3 is n-Ser-Asn-Ile-His-Trp-Cys-Asn-c (SEQ ID NO:19);

R^2 is n-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:11);and R^3 is n-Ser-Asn-Ile-His-Trp-Cys-Asn-c (SEQ ID NO:19);

R^2 is n-Thr-Cys-Val-Ser-c (SEQ ID NO:15); and R^3 is n-Ser-Asn-Ile-His-Trp-Cys-Asn-c (SEQ ID NO:19);

R^2 is n-Thr-Cys-Val-Ser-c (SEQ ID NO:15); and R^3 is n-Ser-Asn-Ile-His-c; (SEQ ID NO:16)

R^2 is n-Ser-c and R^3 is n-Ser-Asn-Ile-His-c (SEQ ID NO:16); or

R^2 is n-Ser-c and R^3 is n-Ser-c; R^1 is Ala.

In another aspect, the invention features a uPA peptide, preferably a purified peptide, which includes a sequence of the formula: n-R^2-Asn-R^1-Tyr-Phe-R^3-c, wherein,

R^1 is an amino acid residue other than L-lysine;

R^2 is a sequence of between 1 and 21 residues in length, having as its carboxy-terminus Ser^{21} of uPA and extending, inclusive of Ser^{21}, from between 1 and 21 amino acid residues in the N-terminal direction of uPA; and

R^3 is a sequence of between 1 and 25 residues in length having as its amino-terminus Ser^{26} of uPA and extending, inclusive of Ser^{26}, from between 1 and 25 amino acid residues in the C-terminal direction of uPA;

wherein c indicates the carboxy terminal direction of the peptide and n indicates the amino terminal direction of the peptide.

In preferred embodiments: R^1 is an amino acid having a side chain with a net-positive charge at a physiological pH, e.g., at pH 7; R^1 is a basic amino acid; R^1 is any of arginine, histidine, or a positively charged or basic non-naturally occurring amino acid.

In preferred embodiments: R^2 is any of:

n-Asp-Cys-Leu-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:9);
n-Cys-Leu-Asn-Gly-Gly-Thr-Cys-Val-Ser-c; (SEQ ID NO:10)
n-Leu-Asn-Gly-Gly-Thr-Cys-Val-Ser-c; (SEQ ID NO:11)
n-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:12);
n-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:13);
n-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:14);
n-Thr-Cys-Val-Ser-c (SEQ ID NO:15);
n-Cys-Val-Ser-c;
n-Val-Ser-c; or
n-Ser-c

In preferred embodiments: R^3 is any of:

n-Ser-c;
n-Ser-Asn-c;
n-Ser-Asn-Ile-c;
n-Ser-Asn-Ile-His-c (SEQ ID NO:16);
n-Ser-Asn-Ile-His-Trp-c (SEQ ID NO:17);

n-Ser-Asn-Ile-His-Trp-Cys-c (SEQ ID NO:18); or
n-Ser-Asn-Ile-His-Trp-Cys-Asn-c (SEQ ID NO:19).

In preferred embodiments: R^2 is n-Asp-Cys-Leu-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:9) and R^3 is n-Ser-Asn-Ile-His-Trp-Cys-Asn-c (SEQ ID NO:19);

R^2 is n-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:11);and R^3 is n-Ser-Asn-Ile-His-Trp-Cys-Asn-c (SEQ ID NO:19);

R^2 is n-Thr-Cys-Val-Ser-c (SEQ ID NO:15); and R^3 is n-Ser-Asn-Ile-His-Trp-Cys-Asn-c (SEQ ID NO:19);

R^2 is n-Thr-Cys-Val-Ser-c (SEQ ID NO:15); and R^3 is n-Ser-Asn-Ile-His-c; (SEQ ID NO:16)

R^2 is n-Ser-c and R^3 is n-Ser-Asn-Ile-His-c (SEQ ID NO:16); or

R^2 is n-Ser-c and R^3 is n-Ser-c; R^1 is Arg.

In another aspect, the invention features a uPA peptide, preferably a purified peptide, including a sequence of the formula: n-R^2-Asn-R^1-Tyr-Phe-R^3-c, wherein,

R^1 is an amino acid residue other than L-lysine;

R^2 is a sequence of between 1 and 21 residues in length, having as its carboxy-terminus Ser^{21} of uPA and extending, inclusive of Ser^{21}, from between 1 and 21 amino acid residues in the N-terminal direction of uPA; and

R^3 is a sequence of between 1 and 25 residues in length having as its amino-terminus Ser^{26} of uPA and extending, inclusive of Ser^{26}, from between 1 and 25 amino acid residues in the C-terminal direction of uPA;

wherein c indicates the carboxy terminal direction of the peptide and n indicates the amino terminal direction of the peptide.

In preferred embodiments: R^1 is an amino acid having a side chain with a net-negative charge at a physiological pH, e.g., at pH 7; R^1 is an amino acid having a side chain which is more negatively charged than is the side chain of lysine; R^1 is an acidic amino acid; R^1 is any of aspartic acid, glutamic acid, or a negatively charged or acidic non-naturally occurring amino acid.

In preferred embodiments: R^2 is any of:

n-Asp-Cys-Leu-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:9);
n-Cys-Leu-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:10);
n-Leu-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:11);
n-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:12);
n-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:13);
n-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:14);
n-Thr-Cys-Val-Ser-c (SEQ ID NO:15);

n-Cys-Val-Ser-c;
n-Val-Ser-c; or
n-Ser-c

In preferred embodiments: R^3 is any of:
n-Ser-c;
n-Ser-Asn-c;
n-Ser-Asn-Ile-c;
n-Ser-Asn-Ile-His-c (SEQ ID NO:16);
n-Ser-Asn-Ile-His-Trp-c (SEQ ID NO:17);
n-Ser-Asn-Ile-His-Trp-Cys-c (SEQ ID NO:18); or
n-Ser-Asn-Ile-His-Trp-Cys-Asn-c (SEQ ID NO:19).

In preferred embodiments: R^2 is n-Asp-Cys-Leu-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:9) and R^3 is n-Ser-Asn-Ile-His-Trp-Cys-Asn-c (SEQ ID NO:19);

R^2 is n-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:);and R^3 is n-Ser-Asn-Ile-His-Trp-Cys-Asn-c (SEQ ID NO:19);

R^2 is n-Thr-Cys-Val-Ser- (SEQ ID NO:15)c; and R^3 is n-Ser-Asn-Ile-His-Trp-Cys-Asn-c (SEQ ID NO:19);

R^2 is n-Thr-Cys-Val-Ser-c (SEQ ID NO:15); and R^3 is n-Ser-Asn-Ile-His-c (SEQ ID NO:16);

R^2 is n-Ser-c and R^3 is n-Ser-Asn-Ile-His-c (SEQ ID NO:16); or

R^2 is n-Ser-c and R^3 is n-Ser-c;

R^1 is glutamic acid.

In another aspect, the invention features a therapeutic composition including a uPA peptide of the invention and a pharmaceutically-acceptable carrier.

In preferred embodiments: the peptide is mitogenic for cells bearing the uPAR; the peptide inhibits mitogenic activity in cells bearing the uPAR; the peptide binds to the uPAR but is not mitogenic for cells bearing the uPAR; the peptide is mitogenic for cells bearing cell surface heparan sulfate proteoglycans, e.g., syndecans, e.g., a syndecan-1, 2, 3 or 4, or any combination thereof.

In preferred embodiments, the uPA peptide modulates the growth or mitogenesis of a proliferating cell to a greater extent than it does a cell of the same type which is proliferating at a lesser rate or is not proliferating.

In another aspect, the invention features a method of regulating, e.g., promoting or inhibiting, the growth or proliferation of a cell, e.g., a cell expressing the urokinase plasminogen activator receptor and/or cell surface heparan sulfate proteoglycans, e.g., syndecans, e.g., a syndecan-1, 2, 3 or 4, or any combination thereof, e.g.. an epidermal cell,

e.g., a keratinocyte, or an osteoblast. The method includes administering to the cell a growth regulating amount of a uPA peptide the invention.

In preferred embodiments: the peptide is mitogenic for cells bearing the uPAR; the peptide inhibits mitogenic activity in cells bearing the uPAR; the peptide binds to the uPAR but is not mitogenic for cells bearing the uPAR; the peptide is mitogenic for cells bearing cell surface heparan sulfate proteoglycans, e.g., syndecans, e.g., a syndecan-1, 2, 3 or 4, or any combiation thereof.

In preferred embodiments, the uPA peptide modulates the growth or mitogenesis of a proliferating cell to a greater extent than it does a cell of the same type which is proliferating at a lesser rate or is not proliferating.

In preferred embodiments, the uPA peptide promotes cell growth or proliferation and the method further includes administering a growth promoting compound, other than a uPA peptide. The compound can be, e.g., a peptide growth factor, e.g., epidermal growth factor or insulin, a complex mixture or extract, e.g., pituitary extract, or a non-peptide compound, e.g., hydrocortisone.

In another aspect, the invention features, a method of regulating, e.g., promoting or inhibiting, the growth or proliferation of epidermal tissue in a patient which has been subjected to trauma, e.g., trauma arising from a disease, e.g., a disease producing an ulceration of epidermal tissue, a surgical incision, a wound, e.g., from a mechanical injury, e.g., a cut, or a burn. The method includes administering to the tissue a growth regulating amount of a uPA peptide of the invention.

In preferred embodiments: the peptide is mitogenic for cells bearing the uPAR; the peptide inhibits mitogenic activity in cells bearing the uPAR; the peptide binds to the uPAR but is not mitogenic for cells bearing the uPAR; the peptide is mitogenic for cells bearing cell surface heparan sulfate proteoglycans, e.g., syndecans, e.g., a syndecan-1, 2, 3 or 4, or any combination thereof.

In preferred embodiments, the uPA peptide modulates the growth or mitogenesis of a proliferating cell to a greater extent than it does a cell of the same type which is proliferating at a lesser rate or is not proliferating.

In preferred embodiments, the uPA peptide promotes cell growth or proliferation and the method further includes administering a growth promoting compound, other than a uPA peptide. The compound can be, e.g., a peptide growth factor, e.g., epidermal growth factor or insulin, a complex mixture or extract, e.g., pituitary extract, or a non-peptide compound, e.g., hydrocortisone.

In another aspect, the invention features a method of regulating, e.g., promoting or inhibiting, the growth of cells, e.g., a sheet of cells, e.g., a sheet of epidermal cells, e.g., keratinocytes, in vitro. The method includes culturing the cells in the presence of an effective amount of a uPA peptide of the invention.

In preferred embodiments: the peptide is mitogenic for cells bearing the uPAR; the peptide inhibits mitogenic activity in cells bearing the uPAR; the peptide binds to the uPAR but is not mitogenic for cells bearing the uPAR; the peptide is mitogenic for cells bearing cell surface heparan sulfate proteoglycans, e.g., syndecans, e.g., a syndecan-1, 2, 3 or 4, or any combination thereof.

In preferred embodiments, the uPA peptide modulates the growth or mitogenesis of a proliferating cell to a greater extent than it does a cell of the same type which is proliferating at a lesser rate or is not proliferating.

In preferred embodiments, the uPA peptide promotes cell growth or proliferation and the method further includes administering a growth promoting compound, other than a uPA peptide. The compound can be, e.g., a peptide growth factor, e.g., epidermal growth factor or insulin, a complex mixture or extract, e.g., pituitary extract, or a non-peptide compound, e.g., hydrocortisone.

In another aspect, the invention features, a method for treating an area of denuded skin in a patient, arising, e.g., from a burn, a wound, or a surgical procedure. The method includes applying cells, e.g., epidermal cells, e.g., a sheet of epidermal cells, produced according to a method of the invention to allow effective attachment of the cells to the underlying dermis of the patient. The method can include administering a uPA peptide, e.g., a growth promoting peptide of the invention, to the patient before or after the sheet is applied to the patient.

In preferred embodiments, the uPA peptide promotes cell growth or proliferation and the method further includes administering a growth promoting compound, other than a uPA peptide. The compound can be, e.g., a peptide growth factor, e.g., epidermal growth factor or insulin, a complex mixture or extract, e.g., pituitary extract, or a non-peptide compound, e.g., hydrocortisone.

In another aspect, the invention includes, a method of identifying an antagonist of uPA, e.g., of the mitogenic activity of urokinase plasminogen activator. The method includes: culturing cells, e.g., uPAR bearing cells and/or a cell surface heparan sulfate proteoglycan, e.g., a syndecan, e.g., a syndecan-1, 2, 3 or 4, or any combination thereof, bearing cells, e.g., epidermal cells, in the presence of a uPA peptide of the invention; contacting the cells with a candidate compound; and comparing the level of mitogenic activity in the presence of the candidate compound to the level of mitogenic activity in the absence of the candidate compound, a lower level of activity in the presence of the compound being indicative that the compound is an antagonist. The candidate compound can be, e.g., an antibody, preferably a monoclonal antibody, e.g., an antibody to the uPAR, or a peptide. Mitogenic activity can be measured, e.g., by the level of [^{3}H]-thymidine incorporation.

In another aspect, the invention includes, a method of identifying an agonist of uPA, e.g., of the mitogenic activity of urokinase plasminogen activator. The method includes: culturing cells, e.g., uPAR bearing cells and/or a cell surface heparan sulfate proteoglycan,

e.g., a syndecan, e.g., a syndecan-1, 2, 3 or 4, or any combination thereof, bearing cells, e.g., epidermal cells, in the presence of a uPA peptide, e.g., a uPA peptide which inhibits mitiogenic activity, contacting the cells with a candidate compound; and comparing the level of mitogenic activity, in the presence of the candidate compound to the level of mitogenic activity in the absence of the candidate compound, a higher level of activity in the presence of the compound being indicative that the compound is an agonist.

In another aspect, the invention features, a method of inhibiting the interaction of urokinase plasminogen activator with urokinase plasminogen activator receptor. The method includes contacting the receptor with a uPA peptide of the invention to inhibit the interaction.

In preferred embodiments: the peptide is mitogenic for cells bearing the uPAR; the peptide inhibits mitogenic activity in cells bearing the uPAR; the peptide binds to the uPAR but is not mitogenic for cells bearing the uPAR; the peptide is mitogenic for cells bearing cell surface heparan sulfate proteoglycans, e.g., syndecans, e.g., a syndecan-1, 2, 3 or 4, or any combination thereof.

In preferred embodiments, the uPA peptide modulates the growth or mitogenesis of a proliferating cell to a greater extent than it does a cell of the same type which is proliferating at a lesser rate or is not proliferating.

In another aspect, the invention features, a method of inhibiting the binding of urokinase plasminogen activator to a cell which expresses uPAR . The method includes contacting the cell with a uPA peptide of the invention to inhibit the interaction.

In preferred embodiments: the peptide is mitogenic for cells bearing the uPAR; the peptide inhibits mitogenic activity in cells bearing the uPAR; the peptide binds to the uPAR but is not mitogenic for cells bearing the uPAR; the peptide is mitogenic for cells bearing cell surface heparan sulfate proteoglycans, e.g., syndecans, e.g., a syndecan-1, 2, 3 or 4, or any combination thereof.

In preferred embodiments, the uPA peptide modulates the growth or mitogenesis of a proliferating cell to a greater extent than it does a cell of the same type which is proliferating at a lesser rate or is not proliferating.

In another aspect, the invention features, a method of determining the growth stage of a cell, e.g., a keratinocyte, including determining the level (e.g., by the use of an antibody or a nucleic acid probe) of urokinase plasminogen activator receptor expressed by the cell.

In another aspect, the invention features, a method for treating an animal having a disorder, e.g., a disorder characterized by an unwanted proliferation of cells, e.g., an unwanted proliferation of uPAR-bearing cells and/or cell surface heparan sulfate proteoglycan, e.g., a syndecan, e.g., a syndecan-1, 2, 3 or 4, or any combination thereof, bearing cells, e.g., an epidermal disorder, e.g., psoriasis, or cancer. The method includes: identifying an animal, e.g., a human, at risk for the disorder; and administering a therapeutically-effective amount of a uPA peptide of the invention to the animal.

In preferred embodiments: the peptide inhibits mitogenesis of uPAR bearing cells; the peptide is mitogenic for cells bearing the uPAR; the peptide binds to the uPAR but is not mitogenic for cells bearing the uPAR; the peptide is mitogenic for cells bearing cell surface heparan sulfate proteoglycans, e.g., syndecans, e.g., a syndecan-1, 2, 3 or 4, or any combination thereof.

In preferred embodiments, the uPA peptide modulates the growth or mitogenesis of a proliferating cell to a greater extent than it does a cell of the same type which is proliferating at a lesser rate or is not proliferating.

In preferred embodiments, the uPA peptide promotes cell growth or proliferation and the method further includes administering a growth promoting compound, other than a uPA peptide. The compound can be, e.g., a peptide growth factor, e.g., epidermal growth factor or insulin, a complex mixture or extract, e.g., pituitary extract, or a non-peptide compound, e.g., hydrocortisone.

In another aspect, the invention features, a method for mitogenically stimulating a cell bearing a uPAR and/or cell surface heparan sulfate proteoglycans, e.g., syndecans, e.g., a syndecan-1, 2, 3 or 4, or any combination thereof, e.g., a keratinocytic cell, including contacting the cell with an effective amount of a uPA peptide of the invention e.g., a fragment of the ATF of uPA having at least six contiguous residues from the GFD.

In another aspect, the invention features, a method for inhibiting mitogenesis in a cell bearing the uPAR, e.g., a keratinocytic cell, including contacting the cell with an effective amount of a uPA peptide of the invention e.g., a fragment of the ATF of uPA having at least six contiguous residues from the GFD.

In another aspect, the invention features, a method of inhibiting the interaction of urokinase plasminogen activator with urokinase plasminogen activator receptor on a keratinocyte including contacting the receptor with an antibody to the urokinase plasminogen activator receptor to inhibit the interaction.

In another aspect, the invention features, a method for inhibiting the growth or proliferation of a cell bearing a uPAR, e.g., a dermal cell, e.g., a keratinocytic cell, including contacting the cell with an antibody to the urokinase plasminogen activator receptor to inhibit growth.

In another aspect, the invention features, a method of modulating, e.g., decreasing, cell proliferation including inhibiting the formation or activity of an ATF fragment of uPA.

In preferred embodiments, the formation or activity of an ATF fragment of uPA is decreased by contacting a cell, e.g., a dermal cell, e.g., a keratinocyte, with an antibody to the urokinase plasminogen activator; by contacting a cell, e.g., a dermal cell, e.g., a keratinocyte, with heparitinase or heparin; by contacting a cell, e.g., a dermal cell, e.g., a keratinocyte, with a plasminogen activator inhibitor type-2 (PAI2); by contacting a cell, e.g., a dermal cell, e.g., a keratinocyte, with a plasminogen activator inhibitor type-1 (PAI1); by contacting a cell, e.g., a dermal cell, e.g., a keratinocyte, with a positively charged lysine homolog.

In another aspect, the invention features a method of modulating, e.g., enhancing or inhibiting, uPA autolysis, including contacting a cell, e.g., a dermal cell, e.g., a keratinocyte, with an effective amount of an autolysis modulating substance.

In preferred embodiments, the autolysis modulating substance is any of: uPA peptide of the invention, e.g., a fragment of the ATF of uPA having at least six contiguous residues from the GFD; an antibody to the urokinase plasminogen activator; a plasminogen activator inhibitor type-2 (PAI2); or a plasminogen activator inhibitor type-1 (PAI1).

In another aspect, the invention features a method of modulating, e.g., enhancing or inhibiting, the binding of uPA or a fragment thereof to a cell surface heparan sulfate proteoglycan, e.g., a syndecan, e.g., a syndecan-1, 2, 3 or 4, or any combination thereof, including contacting a cell, e.g., a dermal cell, e.g., a keratinocyte, with an effective amount of a uPA peptide of the invention, e.g., a fragment of the ATF of uPA having at least six contiguous residues from the GFD.

In another aspect, the invention features, a method of modulating, e.g. enhancing or inhibiting, production of an ATF fragment of uPA, e.g., by modulating, e.g., enhancing or inhibiting, the rate of uPA autolysis.

In another aspect, the invention features, a method for modulating, e.g., promoting, the growth, healing, or adhesion attachment, of a cell bearing a cell surface heparin sulfate proteoglycan, e.g., a syndecan, e.g., a syndecan-1, 2, 3, or 4, or any combination thereof, including contacting the cell with an ATF, provided one or more of the following:

the cell is other than a keratinocyte;

the cell is other than an osteoblast;

the cell is other than a dermal cell;

the cell and the ATF are contacted in vivo.

In preferred embodiments: the cell is an animal cell, e.g., a mammalian cell, e.g., a rodent cell, e.g., a rat or a mouse cell, or a primate cell, e.g., a human cell, and the cell is contacted with the ATF in vivo. The ATF can be delivered, e.g., by topical, parenteral, subcutaneous, intravenous, or intramuscular administration.

In another aspect, the invention features, a method for modulating, e.g., inhibiting, the growth, healing, or adhesion attachment, of a cell bearing a cell surface heparin sulfate proteoglycan, e.g., a syndecan, e.g., a syndecan-1, 2, 3, or 4, or any combination thereof, by inhibiting the binding of an ATF to a syndecan on the cell, provided one or more of the following:

the cell is other than a keratinocyte;

the cell is other than an osteoblast;

the cell is other than a dermal cell;

the cell and the ATF are contacted in vivo.

In preferred embodiments: the cell is an animal cell, e.g., a mammalian cell, e.g., a rodent cell, e.g., a rat or a mouse cell, or a primate cell, e.g., a human cell, and the cell is

contacted with the ATF in vivo. The ATF can be delivered, e.g., by topical, parenteral, subcutaneous, intravenous, or intramuscular administration.

In another aspect, the invention features a method of delivering a compound, e.g., a toxin molecule, e.g., a peptide toxin, to a uPAR and/or a cell surface heparan sulfate porteoglycan, e.g., a syndecan, e.g., a syndecan-1, 2, 3 or 4, or any combination thereof, bearing cell including providing a chimeric molecule which includes the compound coupled, e.g., by a covalent bond, e.g., by a peptide bond, to a fragment of the ATF of uPA at least six residues in length, e.g., to a uPA peptide disclosed herein.

In another aspect, the invention features, a method of inhibiting the proteolytic destruction of an extracellular protein matrix which includes cells bearing the uPAR, e.g., basement membrane, including contacting cells bearing the uPAR with a uPA peptide of the invention.

In preferred embodiments, the uPA peptide promotes cell growth or proliferation and the method further includes administering a growth promoting compound, other than a uPA peptide. The compound can be, e.g., a peptide growth factor, e.g., epidermal growth factor or insulin, a complex mixture or extract, e.g., pituitary extract, or a non-peptide compound, e.g., hydrocortisone.

In preferred embodiments: the peptide inhibits mitogenesis in uPAR bearing cells; the peptide is mitogenic for cells bearing the uPAR; the peptide binds to the uPAR but is not mitogenic for cells bearing the uPAR; the peptide is mitogenic for cells bearing cell surface heparan sulfate proteoglycans, e.g., syndecans, e.g., a syndecan-1, 2, 3 or 4, or any combination thereof.

In preferred embodiments, the uPA peptide modulates the growth or mitogenesis of a proliferating cell to a greater extent than it does a cell of the same type which is proliferating at a lesser rate or is not proliferating.

In another aspect, the invention features, a purified DNA comprising a sequence encoding a uPA peptide of the invention; a vector including a DNA sequence encoding a peptide of the invention; a cell containing the purified DNA, e.g., a cell capable of expressing peptide; an essentially homogeneous population of cells, each of which comprises the isolated DNA; a recombinantly produced peptide of the invention; and a method for manufacture of a peptide of the invention including culturing the cell in a medium to express a peptide of the invention.

The inventors have also discovered that lysine, and analogs of lysine, stimulate mitogenesis of uPAR bearing cells. Accordingly, in another aspect, the invention features a method of regulating growth of a cell, e.g., a uPAR and/or a cell surface heparan sulfate proteoglycan, e.g., a syndecan, e.g., a syndecan-1, 2, 3 or 4, or any combination thereof, bearing cell, e.g., a keratinocyte in vitro, or in vivo, including contacting the cell with, e.g., topically administering to the cell, a growth regulating amount of lysine, or an analog of

lysine, e.g., epsilon-amino caproic acid or tranexamic acid (trans-4-(amino methyl) cyclohexane carboxylic acid.

In preferred embodiments: the lysine or analog thereof is provided at a concentration greater than is provided in growth medium used to culture said cells; lysine is provided at a concentration greater than found in fetal calf or bovine serum.

In other preferred embodiments: the cell is an animal cell, e.g., a human cell, e.g., a keratinocyte the lysine or analog thereof is contacted with the cell in situ, i.e., when the cell is part of the animal and: the concentration of lysine or an analog thereof at the surface of the cell is greater than the highest concentration that can be achieved at the surface of the cell by oral, intravenous, or other systemic administration of lysine or an analog thereof without deleterious effect on the animal; the concentration of lysine or an analog at the surface of the cell is greater than the highest concentration that is achieved at the surface of the cell when lysine or an analog thereof is administered by oral, intravenous, or other systemic administration for nutritional purposes; the lysine or analog thereof is administered to the animal at a concentration amount or dosage higher than the highest concentration, amount, or dosage that can be administered by oral, intravenous, or other systemic administration without deleterious effect on the animal; the lysine or analog thereof is administered to the animal at a concentration, amount, or dosage higher than the highest concentration, amount, or dosage that is administered by oral, intravenous, or other systemic administration of lysine or an analog thereof for nutritional purposes; sufficient lysine is administered such that the concentration of lysine in the dermis is higher, e.g., at least 20, 50, 80, 200, or 400% higher than the concentration of lysine in the dermis of a normal individual or of an individual receiving intravenous nutrition.

In another aspect, the invention features a method of regulating growth of epidermal tissue in a patient which has been subjected to trauma including administering, e.g., topically administering, e.g., to the traumatized tissue, a growth regulating amount of lysine, or an analog of lysine, e.g., epsilon-amino caproic acid or tranexamic acid (trans-4-(amino methyl) cyclohexane carboxylic acid.

In preferred embodiments: the concentration of lysine or an analog thereof at the surface of a cell in the treated tissue is greater than the highest concentration that can be achieved at the surface of a cell in the treated tissue by oral, intravenous, or other systemic administration of lysine or an analog thereof without deleterious effect on the animal; the concentration of lysine or an analog the surface of a cell in the treated tissue is greater than the highest concentration that is achieved at the surface of a cell in the treated tissue when lysine or an analog thereof is administered by oral, intravenous, or other systemic administration for nutritional purposes; the lysine or analog thereof is administered to the animal at a concentration, amount, or dosage higher than the highest concentration, amount, or dosage that can be administered by oral, intravenous, or other systemic administration without deleterious effect on the animal; the lysine or analog thereof is administered to the

animal at a concentration, amount, or dosage higher than the highest concentration, amount, or dosage that is administered by oral, intravenous, or other systemic administration for nutritional purposes; sufficient lysine is administered such that the concentration of lysine in the dermis is higher, e.g., at least 20, 50, 80, 200, or 400% higher than the concentration of lysine in the dermis of a normal individual or of an individual receiving intravenous nutrition.

In another aspect, the invention features a method of regulating the growth of cells, e.g., uPAR bearing cells and/or a cell surface heparan sulfate proteoglycan, e.g., a syndecan, e.g., a syndecan-1, 2, 3 or 4, or any combination thereof, bearing cells, e.g., epidermal cells, e.g., keratinocytes, *in vitro* including culturing the cells in the presence of lysine, or an analog of lysine, e.g., epsilon-amino caproic acid or tranexamic acid (trans-4-(amino methyl) cyclohexane carboxylic acid. In preferred embodiments the lysine or analog thereof is provided at a higher concentration than is lysine or an analog thereof in media used to culture, wash, or otherwise treat the cells; lysine is present in greater concentration than is found in fetal or bovine calf serum.

In another aspect, the invention features a method for treating an area of denuded skin in a patient comprising applying cells, e.g., uPAR bearing cells and/or a cell surface heparan sulfate proteoglycan, e.g., a syndecan, e.g., a syndecan-1, 2, 3 or 4, or any combination thereof, bearing cells, e.g., epidermal cells, e.g., keratinocytes, produced according to a method described herein to allow effective attachment of the cells to the underlying dermis.

In preferred embodiments: the method further includes contacting lysine or an analog thereof with the cells after they have been applied to the patient; the concentration of lysine or an analog at the surface of the cells is greater than the highest concentration that can be achieved at the surface of the cells by oral, intravenous, or other systemic administration of lysine or an analog thereof without deleterious effect on the patient; the concentration of lysine or analog thereof at the surface of the cells is greater than the highest concentration that is achieved at the surface of the cells when lysine or an analog thereof is administered by oral, intravenous, or other systemic administration of lysine or an analog thereof for nutritional purposes; the lysine or analog thereof is administered to the patient at a concentration, amount, or dosage higher than the highest concentration amount or dosage that can be administered by oral, intravenous, or other systemic administration without deleterious effect on the patient; the lysine or analog thereof is administered to the patient at a concentration, amount, or dosage higher than the highest concentration, amount, or dosage that is administered by oral, intravenous, or other systemic administration for nutritional purposes; sufficient lysine is administered such that the concentration of lysine in the dermis is higher, e.g., at least 20, 50, 80, 200, or 400% higher than the concentration of lysine in the dermis of a normal individual or of an individual receiving intravenous nutrition.

In another aspect, the invention features, a method of promoting the growth of a cell, e.g., cell bearing a uPAR receptor and/or a cell bearing a cell surface heparan sulfate proteoglycan, e.g., a syndecan, e.g., a syndecan-1, 2, 3 or 4, or any combination thereof e.g., a

dermal cell, e.g., a keratinocyte, which has been transferred to a site, e.g., the site of a disorder, e.g., a skin disorder or a wound, on a patient including providing the cell, applying the cell to the site, and administering to the patient, e.g., by topical application to the site, a growth promoting amount of lysine or an analog of lysine, e.g., epsilon-amino caproic acid or tranexamic acid (trans-4-(amino methyl) cyclohexane carboxylic acid.

In preferred embodiments: the lysine or analog thereof is provided at a concentration greater than is provided in growth medium used to culture said cells; lysine is provided at a concentration greater than found in fetal calf or bovine serum.

In other preferred embodiments: the concentration of lysine or an analog thereof at the surface of the cell is greater than the highest concentration that can be achieved at the surface of the cell by oral, intravenous, or other systemic administration without deleterious effect on the patient; the concentration of lysine an analog thereof at the surface of the cell is greater than the highest concentration that is achieved at the surface of the cell when lysine an analog thereof is administered by oral, intravenous, or other systemic administration for nutritional purposes; the lysine is administered to the patient at a concentration, amount, or dosage higher than the highest concentration, amount, or dosage that can be administered by oral, intravenous, or other systemic administration without deleterious effect on the patient; the lysine an analog thereof is administered to the patient at a concentration, amount, or dosage higher than the highest concentration, amount, or dosage that is administered by oral, intravenous, or other systemic administration for nutritional purposes; sufficient lysine is administered such that the concentration of lysine in the dermis is higher, e.g., at least 20, 50, 80, 200, or 400% higher than the concentration of lysine in the dermis of a normal individual or of an individual receiving intravenous nutrition.

In another aspect, the invention features a method of identifying an antagonist of the mitogenic activity of urokinase plasminogen activator including culturing uPAR and/or cell surface heparan sulfate proteoglycan, e.g., syndecan, e.g., syndecan-1, 2, 3 or 4, or any combination thereof, bearing cells in the presence of lysine, or an analog of lysine, e.g., epsilon-amino caproic acid or tranexamic acid (trans-4-(amino methyl) cyclohexane carboxylic acid, contacting said cells with a candidate compound, and comparing the level of mitogenic activity in the presence of the candidate compound to the level of mitogenic activity in the absence of the candidate compound, a lower level of said activity in the presence of the compound being indicative that the compound is an antagonist.

In another aspect, the invention features a method identifying an agonist of urokinase plasminogen activator comprising, culturing uPAR and/or cell surface heparan sulfate proteoglycan, e.g., syndecan, e.g., syndecan-1, 2, 3 or 4, or any combination thereof, bearing cells in the presence of lysine, or an analog of lysine, e.g., epsilon-amino caproic acid or tranexamic acid (trans-4-(amino methyl) cyclohexane carboxylic acid, contacting the cells with a candidate compound; and comparing the level of mitogenic activity, in the presence of the candidate compound to the level of mitogenic activity in the absence of the candidate

compound, a higher level of activity in the presence of the compound being indicative that the compound is an agonist.

In another aspect, the invention features a method of inhibiting the interaction of urokinase plasminogen activator with urokinase plasminogen activator receptor including contacting the receptor with lysine, or an analog of lysine, e.g., epsilon-amino caproic acid or tranexamic acid (trans-4-(amino methyl) cyclohexane carboxylic acid to inhibit the interaction.

In another aspect, the invention features a method for treating an animal having a disorder including identifying an animal at risk for the disorder; and administering, e.g., topically, a therapeutically-effective amount of lysine, or an analog of lysine, e.g., epsilon-amino caproic acid or tranexamic acid (trans-4-(amino methyl) cyclohexane carboxylic acid to the animal.

In preferred embodiments: the concentration, amount, or dosage of lysine or an analog administered to the animal results in a concentration of lysine or an analog thereof at the surface of a keratinocyete of the animal that is greater than the highest concentration that can be achieved at the surface of the cell by oral, intravenous, or other systemic administration without deleterious effect on the animal; the concentration, amount, or dosage of lysine or an analog thereof administered to the animal results in a concentration of lysine or an analog thereof at the surface of a keratinocyte of the animal greater than the highest concentration that is achieved at the surface of the cell when lysine an analog thereof is administered by oral, intravenous, or other systemic administration for nutritional purposes; sufficient lysine is administered such that the concentration of lysine in the dermis is higher, e.g., at least 20, 50, 80, 200, or 400% higher than the concentration of lysine in the dermis of a normal individual or of an individual receiving intravenous nutrition.

In another aspect, the invention features a method for mitogenically stimulating a cell, e.g., a uPAR and/or cell surface heparan sulfate proteoglycan, e.g., syndecan, e.g., syndecan-1, 2, 3 or 4, or any combination thereof, bearing cell, e.g., a keratinocytic, including contacting the cell with an effective amount of lysine, or an analog of lysine, e.g., epsilon-amino caproic acid or tranexamic acid (trans-4-(amino methyl) cyclohexane carboxylic acid.

In preferred embodiments: the lysine or analog thereof is provided at a concentration greater than is provided in growth medium used to culture said cells; lysine is provided at a concentration greater than found in fetal calf or bovine serum.

In another aspect, the invention features a method of delivering a compound to a uPAR and/or cell surface heparan sulfate proteoglycan, e.g., syndecan, e.g., syndecan-1, 2, 3 or 4, or any combination thereof, bearing cell including providing a chimeric molecule which includes the compound coupled to lysine, or an analog of lysine, e.g., epsilon-amino caproic acid or tranexamic acid (trans-4-(amino methyl) cyclohexane carboxylic.

In another aspect, the invention features a method of inhibiting the proteolytic destruction of an extracellular protein matrix which includes cells bearing the uPAR or a cell

surface heparan sulfate proteoglycan, e.g., a syndecan, e.g., a syndecan-1, 2, 3 or 4, or any combination thereof, including contacting cells bearing the uPAR or syndecan with lysine, or an analog of lysine, e.g., epsilon-amino caproic acid or tranexamic acid (trans-4-(amino methyl) cyclohexane carboxylic acid.

In another aspect, the invention features therapeutic composition, e.g., a therapeutic composition suitable for topical application, including as an active ingredient lysine or an analog of lysine, e.g., epsilon-amino caproic acid or tranexamic acid (trans-4-(amino methyl) cyclohexane carboxylic acid, and a pharmaceutically-acceptable carrier.

In preferred embodiments the amount of lysine or an analog in the composition is sufficient that one, two, three, five, or less than ten administrations of the composition to the patient results in: a concentration of lysine or an analog thereof at the surface of a keratinocyete of the patient that is greater than the highest concentration that can be achieved at the surface of the cell by oral, intravenous, or other systemic administration without deleterious effect on the animal; a concentration of lysine or an analog thereof at the surface of a keratinocyete of the patient greater than the highest concentration that is achieved at the surface of the cell when lysine an analog thereof is administered by oral, intravenous, or other systemic administration for nutritional purposes.

Substantially pure or purified DNA is DNA that is not immediately contiguous with both of the coding sequences with which it is immediately contiguous (i.e., one at the 5' end and one at the 3' end) in the naturally-occurring genome of the organism from which the DNA of the invention is derived. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., a cDNA or a genomic DNA fragment produced by PCR or restriction endonuclease treatment) independent of other DNA sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence.

Homologous refers to the sequence similarity between two polypeptide molecules or between two nucleic acid molecules. When a position in both of the two compared sequences is occupied by the same base or amino acid monomeric subunit, e.g., if a position in each of two DNA molecules is occupied by adenine, then the molecules are homologous at that position. The homology between two sequences is a function of the number of matching or homologous positions shared by the two sequences. For example, 6 of 10, of the positions in two sequences are matched or homologous then the two sequences are 60% homologous. By way of example, the DNA sequences ATTGCC and TATGGC share 50% homology.

A substantially pure or purified preparation of a peptide is a preparation which is substantially free of the peptides or proteins with which the peptide (or the protein from which it is derived, e.g., whole length uPA, in the case of synthetic uPA peptides) naturally occurs in a cell.

The inventors have found that the ability to bind to uPAR and the mitogenic activity of uPA is preserved with uPA peptides as small as a 9-mer (GFD 21-29) (i.e., uPA residues 21-29 of the GFD), whose sequence is Ser-Asn-Lys-Tyr-Phe-Ser-Asn-Ile-His ((SEQ ID NO:1), and a 6-mer (GFD 21-26), whose sequence is Ser-Asn-Lys-Tyr-Phe-Ser (SEQ ID NO:2). These peptides can stimulate the growth of keratinocytes without causing side effects of tissue bleeding. The invention allows for promoting the repair of injured tissue by causing epidermal cells to be stimulated to grow and thus repair the wound.

The small size of uPA peptides of the invention, e.g., the 9-mer and the 6-mer, is important in that smaller peptides will more readily penetrate to the basal layer (the layer with mitotoic activity in the normal epidermis) and are more effective in topically stimulating epidermal cells to grow.

Peptides and methods of the invention can be used to stimulate the growth of epidermal sheets of cells in vitro or in vivo. These sheets of cells can be used, e.g., as covering for large areas of denuded skin, such as burns. The invention provides a method for stimulation of the epidermal cells, not only to produce epidermal sheets more rapidly and effectively, but also to stimulate continued growth of the epidermal cells once they are place upon the wounded tissue. The invention will enhance the ability of in vitro grown epidermal sheets applied to a patient to further grow and produce basement membrane components for effective attachment to the underlying dermis.

uPA peptides of the invention are also useful for blocking the binding of uPA or fragments thereof to receptors, to thus inhibit the activity of uPA. uPA peptides having mitogenic activity, uPA peptides which do not stimulate mitogenic activity, or uPA peptides which inhibit mitogenisis, can be used. Peptides having mitogenic activity will bind and allow for cell proliferation without the proteolytic effects of uPA. The peptides not having mitogenic activity will block uPA activity but exhibit neither mitogenic activity nor proteolytic activity. uPA peptides of the invention are aslo useful in modulating uPA catalytic activity.

The inventors have also discovered that the amino acid at residue 23 of uPA plays an important role in the activity of a uPA peptide. As is described below, uPA peptides with enhanced mitogenic activity and uPA peptides with inhibitory properties can be synthesized by replacing the lysine normally found at position 23. Substituted peptides are useful for inhibiting or enhancing the growth or proliferation of cells and for receptor binding studies.

Other features and advantages of the invention will be apparent from the following description and from the claims.

Detailed Description

The drawings will first be briefly described.

Drawings

Fig. 1 is a bar graph which represents the effect of various uPA peptides on DNA synthesis in keratinocytes. Various concentrations of GFD peptides were tested in order to

compare the mitogenic activity. After 24 h incubation with a peptide and ^{3}H-thymidine, incorporated radioactivity was measured as described herein. Five concentrations were used for each peptide. For a given peptide the uppermost bar of the five represents 0.1 μM, the next lower bar 0.33 μM, the next lower bar 1 μM, the next lower bar 3.3 μM, and the lowest bar, 10 μM.

Fig. 2 is a bar graph which represents the additive effect of the 6-mer peptide in different growth media. XBM, keratinocyte basal medium (keratinocyte growth medium without epidermal growth factor, insulin, hydrocortisone, or bovine pituitary extract); KGM-BPE, keratinocyte growth medium without bovine pituitary extract; BM+Ins, keratinocyte basal medium plus insulin; BM+EGF, keratinocyte basal medium plus epidermal growth factor; BM+BPE, keratinocyte basal medium plus bovine pituitary extract; KGM, keratinocyte growth medium (Clonetics, CA).

Fig. 3 is a graphic representation of uPA activity in keratinocyte culture over a 22 day period. The solid line represents total cell number; the dotted line represents total uPA activity in the medium; and the dashed line represents the uPA activity per cell.

Fig. 4 is a schematic diagram of synthetic GFD peptides.

Fig. 5 is a schematic diagram of synthetic 9 mer and 6 mer GFD peptides with modifications at Lys^{23} position.

Fig. 6 is a graphic representation of effect of GFD peptide on keratinocytes. In the early stage of the culture (30% confluent), none of the peptides showed growth stimulation even at the highest concentration (6A). When GFD-9K was added at around 60-80% confluency, considerable increase in thymidine incorporation was observed. GFD-9A greatly enhanced this effect in a dose dependent manner. Neither GFD-9R or GFD-9E demonstrated growth stimulation (6B). After reaching confluency, this stimulation was no longer observed with any peptides at any concentration (6C). Essentially the same results were obtained for 6mer peptides (6D-F).

Fig. 7 is a graphic representation of keratinocyte proliferation and PA activity. Third passage keratinocytes were cultured with serum-free KGM medium in 96-well plates. Every two days, PA activity in the medium (open circle) was measured using plasminogen-supplemented two step assay and expressed as International Unit (IU). After trypsinization, keratinocytes were collected and cell number (closed square) was counted using hemocytomer. PA activity per cell number is also shown (closed circle). Results were mean ± SE for 6 experiments.

Fig. 8 is a graphic representation of cell synchronization and PA activity. Keratinocytes were synchronized into the S phase using thymidine and hydroxyurea. After initiating the new cell cycle by adding fresh KGM medium, ^{3}H-thymidine was incorporated for 30 min and radioactivity in TCA-insoluble materials was measured every 2.5 h. A peak of ^{3}H-thymidine (S phase) was seen around 20 h (17.5-20 h in other experiments). In addition, PA activity was determined by removing 50 μl of culture medium every 2.5 h and

expressed in IU/ml. A peak PA activity was usually observed just prior to the S phase. Results were mean ± standard diviation (SD) for 3 experiments.

Fig. 9 is a graphic representation of the effect of ATF on thymidine incorporation. After autolysis, ATF was isolated from the proteinase domain of uPA by Sephadex G-100 and cation-exchange Mono S chromatogarphy. To the synchronized keratinocytes, 0.1 μM of ATF was added and changes of ^{3}H-thymidine incorporation were monitored. Results were mean ± SD for 3 experiments.

Fig. 10 is a graphic representation of the effect of GFD peptide on keratinocyte proliferation. Keratinocytes were cultured with or without 1 m M GFD peptide which corresponds to 12-32 of the uPA sequence. On the designated days, cells were harvested and cell numbers were counted. Results were mean ± SD for 6 experiments.

Fig. 11 is a graphic representation of A: Effect of antibodies and enzyme treatment on thymidine incorporation. Various factors which affects the growth stimulation by the GFD peptide was investigated using ^{3}H-thymidine incorporation assays. Blocking antibody to uPA or anti-ATF antibody was added to the medium and effect on ^{3}H-thymidine incorporation was assayed after overnight incubation together with various concentration of the GFD peptide. B: In another series of experiments, heparitinase or chondroitinase ABC was added to the medium ^{3}H-thymidine incorporation was studied similarly.

Fig. 12 is a graphic representation of solid phase binding assay with syndecan. After metabolic labeling with $^{35}SO_4^{2-}$, human keratinocyte syndecan was purified by immunoprecipitation using the polyclonal antibody, MS-1-C. This antibody was directed to the cytoplasmic domain of mouse syndecan-1 which recognizes all forms of human syndecans. Various concentration of uPA, ATF, proteinase domain of uPA (LMW uPA) and bovine serum albumin were blotted onto a nitrocellulose membrane and binding activity to syndecan was investigated.

Fig. 13 is a graphic representation of column chromatographic analysis. Recombinant ATF (rATF) was constructed as a glutathione S-transferase fusion protein and coupled to the glutathione-Sepharose affinity column. Purified uPA was also coupled to CNBr-activated Sepharose 4B and packed in a small column. Radio-labeled syndecan was applied to these columns and eluted with 1 M NaCl. Recovered radioactivity was measured in each fraction for rATF (closed circle) and uPA (open circle) affinity columns. In the selected experiments, either10 μg/ml of heparin or 10 m g/ml of chondroitin sulfate B was added to the syndecan sample prior to the application.

Fig. 14 is a schematic diagram of the mechanism of uPA growth stimulation. Primary event is the generation of ATF by autolysis process. This will occur under certain circumstances, where production of uPA is highly elevated as seen in the case of cancer cell and keratinocyte proliferation in culture. Generated ATF undergoes a conformational change and exposes a heparan sulfate binding site, resulting the binding to the cell suface heparan

sulfate proteoglycan, syndecan. Growth signal would be transmitted through cytoplasmic domain-associated molecule(s).

Mitogenic activity of uPA fragments in keratinocytes

uPA is a 55 Kda protein which has an EGF-like domain, a kringle domain, and a trypsin-like protease domain (uPA has been cloned, see Riccio et al., *1985 , Nucleic Acids* Research 13: No. *8).* uPA binds to a specific cell receptor (uPAR) through the EGF-like domain (uPAR has been cloned, see Roldan, et al., 1990 *EMBO Journal* 9:467). The 18 Kda amino terminal region of urokinase which includes the EGF-like domain and the kringle domain has been found to induce mitogenesis in an osteoblast cell line (Rablani et *al .*, *Biochem . Biophys . Res . Commun.* 173:1058, 1990).

In order to obtain small molecules which retained the mitogenic activity of uPA, but which did not have fibrinolytic activity, a number of peptides of various lengths were designed to determine the portion or portions of the uPA molecule which is required for mitogenic activity. The synthesis and analysis of these peptides is described below.

Preparation of Peptides Synthetic peptides of various lengths were synthesized according to standard methods using a peptide synthesizer (Research Genetics, Inc.). Peptides were purified by reverse phase HPLC (Toso, Inc.). The amino acid sequences are given in Table 1.

Mitogenesis Assay The effect of a peptide on mitogenesis was determined as a function of DNA synthesis as follows. Peptides (in phosphate buffered saline (PBS)) were added to the monolayers (grown as described herein) to a final concentration of either 0.1 μ M, 0.33 μM, 1.0 μM, 3.3 μM, or 10 μM. 5 μL of ^{3}H-thymidine (10 μCi/ml; Amersham, Arlington Heights, IL) in 150 μL KGM was added to the cultures concurrent with the peptides, and cells were harvested and lysed after 24 hours at 37°C in distilled H_20 using a Tomtec cell harvester, and fixed with 70% ethanol. The lysed cells were then transferred to a nylon filter (Amersham, Arlington Heights, IL), Beta plate scintillation fluid was added (LKB), and radioactivity was measured using a Wallac 1205 beta plate counter. Control samples, incubated in the presence of phosphate-buffered saline (PBS) and ^{3}H-thymidine gave control levels of radioactivity of approximately 400 cpm.

Cell Culture Primary human keratinocytes (Clonetics, CA) were cultured in 96-well plates (Corning, NY) in serum-free medium (KGM medium, Clonetics) to 70-80% confluency on a monolayer culture at 37°C in 5% C02.

Short uPA Peptides Have Mitogenic Activity Several short uPA peptides were synthesized and tested for mitogenic activity as described herein. The peptides are shown in Table 1.

TABLE 1

Sequence of uPA Peptides

21-mer:	DCLNGGTCVSNKYFSNIHWCN	(Seq ID No 3)
18-mer:	NGGTCVSNKYFSNIHWCN	(Seq ID No 4)

15-mer:	TCVSNKYFSNIHWCN	(Seq ID No 5)
12-mer:	TCVSNKYFSNIH	(Seq ID No 6)
9-mer:	SNKYFSNIH	(Seq ID No 1)
6-mer:	SNKYFS	(Seq ID No 2)

As shown in Fig. 1, all of the peptides tested stimulated thymidine incorporation. The 6-mer peptide did not demonstrate any measurable activity at concentrations up to 3.3 μM, but at a concentration of 10 μM resulted in a significant stimulation of ^{3}H-thymidine incorporation. The 9-mer peptide was effective in stimulating mitogenesis at concentrations as low as 0.33 μLM, with a maximum level of mitogenic activity at 1 μM. The 12-mer demonstrated mitogenic activity only at 1 μM, and had little, if any, effect on keratinocyte stimulation at other concentrations. The highest levels of mitogenic activity produced by the 15-mer occurred at a concentration of 3.3 μM. At a concentration of 3.3 μM and 10 μM, the 21-mer and the 18-mer showed similar effects on the stimulation of mitogenesis in keratinocytes. PBS controls showed approximately 400cpm of incorporation. The entire ATF was also found to stimulate keratinocyte mitogenesis.

The interaction of the 6-mer peptide (GFD 21-26) with other growth factors

The 6-mer peptide (GFD 21-26), was studied further in order to determine its effect on keratinocyte stimulation in the presence or absence of other factors. Keratinocytes were cultured in various growth media which lacked different growth factors which are normally present in KGM (complete medium) for 24 hours in the presence or absence of the 6-mer (Fig. 2), and ^{3}H-thymidine incorporation was measured as described above.

As shown in Fig. 2, the 6-mer (P-8) stimulated ^{3}H-thymidine uptake greatly in the presence of the complete keratinocyte growth medium which contains various growth factors such as bovine pituitary extract (BPE), insulin, EGF, and hydrocortisone. In addition, the 6-Mer always showed additive effect to any growth factors tested in this study.

Expression of urokinase plasminogen activator receptor (uPAR) on proliferating keratinocytes

Northern blot analysis was utilized to determine the level of uPAR expression in keratinocytes at various stages of growth. Briefly, a 0.4 kb probe which encodes the ectodomain of the uPAR was prepared by the RT-PCR technique (Invitrogen, cDNA cycle kit) using the primers: 5'-GGGGATTGCCGTGTGGAAGA-3' (SEQ ID NO: 7) and 5'-GGAATTCGAAGGTAGCCACAGCCACGGAG-3' (SEQ ID NO: 8). Messenger RNA was purified from cultured keratinocytes in monolayer culture at the stages of 50% confluency, 80% confluency, 100% confluency, and after confluency (120% confluency). Approximately 2 μg of purified mRNA from each cultured keratinocyte stage was then subjected to electrophoresis in agarose and transferred to a nylon membrane (Hybond N). After pre-hybridization in 6X SSC, 5X Denhardt's, 0.5% SDS, 100 μg/ml sonicated salmon sperm DNA, and 50% deionized formamide, the [^{32}P]-labeled uPAR probe was added, and hybridization was allowed to proceed at 42°C overnight in the presence of 50% formamide.

The nylon membrane was then washed twice with 2x SSC containing 0.1% SDS, followed by washes in 0.1x SSC, 0.1% SDS at 37°C for 30 mins and then at 55°C for 30 mins. Autoradiography was performed by exposing Kodak XRR film at -70°C.

The data of the Northern blot analysis indicated that uPAR mRNA is highly expressed during stages of exponential growth (e.g., at 50% confluency). In addition, the data also indicate that the expression of uPAR mRNA decreases as confluency increases over 50%, and is almost undetectable at 100% confluency. These results indicate that there is a direct correlation between the expression of the uPAR mRNA and keratinocyte proliferation.

Urokinase plasminogen activator activity in keratinocytes

Normal human keratinocytes (Clonetics, CA) were cultured in 96-well plates using serum free keratinocyte growth medium and cell number was monitored every two days by hemocytometer. uPA activity was also measured every two days using a two-step assay. Briefly, 50 µl of conditioned medium from the keratinocyte culture was mixed with 0.1 M Tris-HCl, pH 8.5, containing 0.1% Tween-20, and 50 µl 0.1 mg/ml purified plasminogen in PBS, and incubated for 30 mins at 30°C. Fifty microliters of 0.2 M phosphate buffer, pH 7.2, containing 1.4 M NaCl mixed with 4 mM of the substrate S-2251 (Val-Leu-Lys-p-nitroanilide; Kabi Diagnostics, Stockholm) to a final concentration of 0.8 mM S-2251, was then added. After incubation, the absorbence of each sample was measured at 405nm, and uPA activity was determined using the International Standard of urokinase.

Fig. 3 summarizes the uPA activity in normal human epithelial keratinocytes. The unbroken line represents the cell number of keratinocytes in culture over a 22 day period, and shows that cell number rapidly increases from day 6, approaches 100% confluency at day 8, then continues to increase after 100% confluency at day 12, and begins to decline at approximately day 14. The dotted line represents the uPA activity over the same 22 day period. Total uPA production increases up to approximately day 12 and then decreases. The dashed line represents the uPA activity per cell and demonstrates that uPA activity per cell increases prior to confluence, and then decreases when confluence is reached. These data further substantiate that there is a direct correlation between uPA activity and keratinocyte proliferation.

uPA peptides with altered activities

Computer analysis of the structure of uPA regions suggested the presence of a turn-structure at Lys^{23} of GFD. The effect of substitutions at Lys^{23} was evaluated in uPA peptides. 9mers and 6mers were synthesized wherein Lys^{23} was replaced by:
alanine, Ser-Asn-Ala-Tyr-Phe-Ser-Asp-Ile-His (SEQ ID NO:20) (A-GFD);
arginine, Ser-Asn-Arg-Tyr-Phe-Ser-Asp-Ile-His (SEQ ID NO:21) (R-GFD); and
glutamic acid, Ser-Asn-Glu-Tyr-Phe-Ser-Asp-Ile-His (SEQ ID NO:22) (E-GFD)
alanine, Ser-Asn-Ala-Tyr-Phe-Ser (SEQ ID NO:23) (A-GFD);
arginine, Ser-Asn-Arg-Tyr-Phe-Ser (SEQ ID NO:24) (R-GFD); and

glutamic acid, Ser-Asn-Glu-Tyr-Phe-Ser (SEQ ID NO:25) (E-GFD) (Fig. 5). The ability of these substituted peptides to stimulate mitogenesis was determined by ^{3}H-thymidine uptake. In the early stage of the culture (approx. 30% confluent), these peptides had little effect on thymidine incorporation (Fig. 6A). At 60-90% confluent condition, the positive control 9-mer Ser-Asn-Lys-Tyr-Phe-Ser-Asp-Ile-His (SEQ ID NO:1) (K-GFD) stimulated thymidine incorporation in a dose dependent manner. (Fig. 6B). A-GFD-9 (SEQ ID NO:20) greatly enhanced this effect. R-GFD (SEQ ID NO:21) and E-GFD (SEQ ID NO:22) peptides did not show any positive effect. After reaching confluent monolayer, growth stimulation was not observed by any peptides. (Fig. 6C)). Essentially the same results were obtained for the GFD-6 peptides. (Fig. 6D-F) These results reveal that the amino acid residue at position 23 plays an important role in keratinocyte stimulation and that this effect is limited to a certain stage of the keratinocyte culture. Other useful peptides can be found by synthesizing uPA peptides, e.g., peptides shown in Table 1 with various substitutions at position 23 (or other positions) and determining their effect on mitogenesis.

Mechanism of keratinocyte growth stimulation by uPA

1. PA activity profile in keratinocyte conditioned medium

PA activity and cell number were determined every other day from keratinocytes cultured in a 96-well plate (Fig. 7). The total PA activity increased up to 12 days of culture. Cell number also reached its maximum on that day and gradually decreased thereafter. Approximately 3 IU/ml of PA activity was maintained between 8 days, when cells nearly reach confluency, and 14 days, when the period of spontaneous shedding begins . The PA activity/cell peaked on the 4th day (4.4 mIU/ml/cell) and sharply declined thereafter, indicating that production and secretion of PA is most prominent in the early stages of culture when keratinocytes enter the exponential growth stage.

Normal human epidermal keratinocytes were prepared from foreskin (Rheinwald and Green (1975) *Cell* 6:331-344) and cultured in a serum-free K-GM medium (Clonetics Co., San Diego, CA). For all experiments described cells were subcultured using 0.25% trypsin + 0.02% EDTA and used before the 3rd passage. .

PA activity in the culture medium was measured by a plasminogen-supplemented two-step assay. 10 to 50 ml of the samples were mixed with 90 to 50 ml of 0.1 M Tris-HCl (pH 8.8) + 0.1% Tween 80 and 50 ml of 0.2 mg/ml of human plasminogen and incubated at 37° C for 30 min. After mixing the sample with 50 ml of 0.2 M phosphate buffer (pH 7.0) containing 0.2 M NaCl, 50 ml of 1 mM synthetic substrate for plasmin (S-2251) was added and incubated for the appropriate times. The reaction was stopped by adding 50 ml of 30% acetic acid and changes of absorbance were measured at 405 nm using an EIA reader. uPA activity was expressed as an International Unit (IU) using urokinase as standard .

2. uPA is the species of PA synthesized by keratinocytes

The species of PA was determined by neutralization studies using monospecific polyclonal antibodies to u-PA and t-PA. 50 ml of conditioned medium were incubated with

anti-uPA or anti-tPA antibody for 30 min at room temperature. The mixture was then centrifuged at 12,000 xg for 20 min and PA activity was measured in the supernatant. Anti-uPA antibody, but not anti-tPA antibody, suppressed most of the PA activity in conditioned medium obtained on the 4th and 10th days of culture. These two time points correspond to the stages of proliferation and confluency respectively. The results indicate that u-PA is secreted throughout the culture period (Table 2).

Goat anti-t-PA was from Biopool AB (Umea, Sweden).

Table 2. Effects of Anti-PA Antibody

Culture Day	Control	+ anti-uPA IgG	+ anti-tPA IgG IU/ml
4	1.45 ± 0.20 (100%)	0.08 ± 0.05 (5.5%)	1.42 + 0.35 (2.0%)
10	2.25 ± 0.15 (100%)	0.15 ± 0.11 (5.9%)	2.45 ± 0.20 (3.9%)

3. Analysis of steady state levels of uPA and uPAR transcripts

Poly(A)$^+$ RNA was isolated from cultured keratinocytes at 50% (day 5), 80% (day 7) and 100% confluency (day 9), and after confluency (day 12). Steady state levels of u-PA mRNA were high until subconfluency and decreased dramatically when keratinocytes reached confluency suggesting that uPA production is closely related to cell proliferation and that contact inhibition may be a turning-off signal for uPA production. Analysis of steady state levels of mRNA for uPAR demonstrated a different pattern from that of uPA in that uPAR transcripts are limited to the earlier growth stage of the culture. Expression of hEF-1a was constant throughout the culture period.

Polyadenylated RNA was isolated using the FastTrack mRNA isolation kit according to the manufacturer's instructions. All samples used for Northern blot analysis had a minimum 260/280 ratio of 1. After electrophoresis on 1% agarose gel containing formaldehyde, mRNA fractions (2 mg each) were transferred onto nylon membranes. The membranes were prehybridized at 45^o C for 1 h in 50% formamide, 6 x SSC, 2 x Denhardt, 0.1% SDS and 200 mg/ml salmon sperm DNA. Hybridization was carried out at 45 ^{o} C for 16 h in the same mixture after adding ^{32}P-labeled probes. The membranes were then washed twice in 2 x SSC with 0.1% SDS for 20 min each at room temperature, twice in 0.1 x SSC with 0.1% SDS for 20 min each at 65^o C, and autoradiographed using XAR film at -70^o C.

The uPAR probe was prepared by reverse transcription followed by PCR using a cDNA Cycle Kit (Invitrogen, San Diego, CA). Briefly, 0.5 mg of the purified mRNA from the cultured keratinocytes was mixed with reverse transcriptase to produce the first strand cDNA. The PCR primers were designed on the basis of the report of Roldan et al. (1990) *EMBO J.* 9:467-474. The sequence for the upper primer was 5'GGGFATTGCCGTGTGGAAGA3' (SEQ ID NO:26) The sequence of lower primer with

an Eco RI site was 5'GGAATTCGAAGGTAGCCACAGCCACGGAG3' (SEQ ID NO:27). After 30 cycles of PCR, a single band of approximately 440 bases, which corresponds to the expected length, was demonstrated in 1.2% agarose (low melting agarose, Sekam, Rockland, ME) gel electrophoresis. The band was cut out and the DNA was extracted twice with phenol, once with phenol:chloroform and precipitated with 100% ethanol.

The uPA probe was derived from plasmid pUK-E1 which contains a 413 bp Eco RI cDNA fragment of uPA. This plasmid was kindly supplied by Dr. H Arimura (Green Cross Co., Osaka, Japan). Plasmid pAN7, which carries a 1.9 Kb Bam HI cDNA fragment of human elongation factor-1a (hEF-1a), was provided by Dr. T. Uezuka and used as control. The purified cDNA fragments were nick-translated in the presence of [a-^{32}P]dCTP to give a specific activity > 10^8 cpm/mg of DNA.

4. Cell Synchronization and PA activity

Cell synchrony was monitored at 2.5 h intervals by measuring radioactivity in TCA-insoluble keratinocyte extracts after 30 min of ^{3}H-thymidine incorporation. The majority of keratinocytes were synchronized by this treatment. The S phase was indicated by a peak of ^{3}H-thymidine uptake between 17.5 and 20 h (Fig. 8). The medium was changed every 2.5 h and the PA activity was measured. The uPA activity changed significantly during the cell cycle with a peak of activity just prior to the S phase. The results imply that production and secretion of uPA is a specific event associated with the cell cycle during keratinocyte proliferation.

Exponentially growing keratinocytes were synchronized into S phase by treatment with thymidine and hydroxyurea (Cidlowski et al. (1982) *Endocrinology* 110:1653-1662); (M.H. Goyns (1980) *Experientia.* 36:936-937) based on a doubling time of 40 h determined in preliminary experiments. Thymidine was added to the NHEK culture to a final concentration of 2.5 mM and incubated for 26 h. After washing twice with PBS, the cells were incubated in fresh K-GM medium for 14 h. Synchrony was accomplished by the addition of hydroxyurea to a final concentration of 1 mM for 24 h. To assess cell synchrony, ^{3}H-thymidine (0.5 m Ci/ml) was incorporated for 30 to 90 min. Cells harvested by trypsin treatment were placed on glass microfiber filters (GF/C, Whatman, Maidstone, England) and radioactivity in TCA-insoluble materials was measured using LS 6000IC counter (Beckman, Palo Alto, CA).

5. Immunohistochemical localization of uPA and ATF

At day 1, all cells are strongly positive for uPA. Since uPA is anchored to the specific uPAR, this staining implies the presence of uPA-uPAR complexes on the cell surface. In 5 day keratinocyte cultures two types of staining patterns for uPA are observed. In one type a continuous line of uPA staining is seen in areas of cell-cell contact, suggesting the occurrence of the uPA-uPAR complex in areas of cell-cell contact. The second type of staining is seen in cells showing mitogenic features and these cells stain uniformly. Some of the peripheral keratinocytes also demonstrated strongly positive staining on the entire cell surface area.

These cells may be in pre-mitotic stage judged by the larger size of their nuclei. This explanation is consistent with the cell synchrony results which showed that uPA is secreted prior to the S phase. The mesh-like staining pattern was not observed on the cell membrane of the non-contact area at the outermost part of colonies, where pseudopodia-like structures are seen with this staining. The difference between the inner cells that show a mesh-like pattern and some of the outer cells whose entire cell surface stains suggests that 1) cell-cell attachment causes condensation of uPA complexes to the contact area and 2) keratinocytes which undergo mitosis possess uPA on the entire cell surface area.

An anti-ATF antibody which is directed against the EGF-like domain of uPA was also used. This antibody cannot recognize the uPAR-bound form of uPA, since uPA binds to uPAR through this domain. Anti-ATF staining of day 5 culture demonstrate a punctate pattern on the cell surface. None of the continuous staining in cell-cell contact areas or on the entire cell surface staining is observed with this antibody. These results suggest that some uPA or most likely ATF localizes not only through uPAR but through other cell surface molecule(s).

To localize the membrane associated uPA and uPAR coverslip cultures were brought to 0° C and washed three times with phosphate-buffered saline (PBS) containing 5 mg/ml BSA (PBS-BSA). The cells were incubated with the mAbs in PBS-BSA for 30 min at 0° C. After washing, the cells were fixed with methanol at -20° C for 20 min. Rhodamine-conjugated anti-mouse antibody was used as a secondary antibody. Nuclear staining was also performed using Hoechst dye. Monoclonal anti-uPA antibody (#394) was used for uPA staining. Monoclonal anti-ATF antibody (#3471) was used to distinguish unbound uPA from the receptor-bound form. Monoclonal antibody to uPA (#394) is directed against the proteinase domain of uPA and recognizes all forms of uPA including pro-uPA and receptor-bound uPA. Monoclonal antibody to ATF (#3471) was produced against the binding sequence (17-34) of ATF and thus recognizes unbound uPA only. The anti-ATF monoclonal antibody was used to distinguish unbound uPA from the receptor-bound form. Both monoclonal antibodies were purchased from American Diagnostica (Greenwich, CT).

To investigate the association of uPA to cell surface heparan sulfate proteoglycan, 30 milliunits of heparitinase, 30 milliunits of chondroitinase ABC, or 0.1 unit of GPI-specific phospholipase C was added to 100 ml of culture medium and incubated overnight at 37° C before carrying out the immunohistochemical tests.

6. ATF stimulates keratinocyte proliferation

After cell synchrony, keratinocytes were kept in a basal medium (K-BM) without growth factors for 15 h. The medium was then changed to K-GM (complete growth medium) and cells were cultured with or without 0.1 mM of ATF. ^{3}H-thymidine uptake was measured after 1.5 h. The control study showed that ^{3}H-thymidine incorporation decreases until the fifth hour and then increases gradually. With the addition of ATF, ^{3}H-thymidine uptake

doubled by the tenth hour compared to the control levels. Thus ATF is capable of stimulating keratinocyte proliferation (Fig. 9).

Human uPA was purified from a commercially available form (Green Cross, Co., Osaka, Japan) using benzamidine affinity chromatography (Holmberg, et al. (1976) *Biochim. Biophys. Acta.* 445:215-222). ATF was prepared from the purified uPA by the method of Stoppelli et al. (1985) *Proc. Natl. Acad. Sci. USA* 82:4939-4943). After autolysis, ATF was isolated by Sephadex G-100 gel chromatography followed by FPLC Mono S cation-exchange chromatography. The yield was 5% of the original starting uPA protein. The final preparation showed a single band with M_r 18,000 on SDS polyacrylamide gel electrophoresis (PAGE).

7. Effect of GFD-21 on keratinocyte proliferation

Although ATF contains both the EGF-like domain and a kringle domain, the EGF-like domain may be the potential growth factor domain. In order to test this hypothesis, a peptide which corresponds to amino acid residues 12-32 from the EGF-like domain of ATF (GFD-21) was synthesized and added to the medium every day to a final concentration of 1 mM. The cell number was determined on days 7, 9 and 11 (Fig. 10). In the presence of the GFD-21 peptide, an increase in cell number was consistently observed after day 7. At day 9 the cell number was increased by approximately 50% indicating that the EGF-like domain of ATF may be involved in the stimulation of proliferation.

A peptide with a sequence corresponding to amino acid residues 12-32 of uPA was custom synthesized by Research Genetics, Huntsville, AL. In this sequence Cys^{19} was replaced by Ala to avoid intermolecular or undesired disulfide bond formation. The sequence of this growth factor domain peptide (GFD-21) was DCLNGGTAVSNKYFSNIHWCN. GFD-21 (SEQ ID NO:28) was purified by HPLC using a reverse phase C_{18} column (YMC-Pack, Tosoh Co., Japan). The peptide composition was confirmed by amino acid analysis.

8. Catalytically active uPA is required for stimulating kertinocyte proliferation

To characterize the role of the GFD-21 peptide in keratinocyte proliferation, we used two different antibodies. One antibody neutralizes the protease activity of uPA and the other blocks the interaction between uPA and uPAR. When the protease neutralizing antibody was added to the medium, keratinocytes proliferation was completely inhibited and the stimulatory of the GFD-21 peptide was abolished (Fig. 11A). These results indicate that the mitogenic effect of the GFD-21 peptide requires catalytically active uPA and that the peptide effect is mediated through uPA activity.

Addition of the anti-ATF antibody to the culture medium had no suppressing effect on keratinocyte proliferation. Since this antibody blocks uPAR binding, the results suggest that the stimulatory effect of the GFD-21 peptide is indirect and not through uPAR. Furthermore, uPA-uPAR complex formation may not initiate the growth stimulation, since anti-ATF antibody, which is capable of blocking the binding of both uPA and ATF to uPAR did not

show any suppressing effect. These results strongly suggest the involvement of a yet unidentified molecule in the proliferative response of keratinocytes to uPA..

9. Heparan sulfate proteoglycan is required for growth stimulation

When keratinocytes are incubated with heparitinase before the addition of the GFD-21 peptides, ^{3}H-thymidine incorporation is also suppressed at all concentration of the GFD peptide (Fig. 11B). Chondroitinase ABC did not suppress ^{3}H-thymidine uptake, showing the specificity of heparitinase treatment. These results also indicate the requirement of a cell surface HSPG for growth stimulation of keratinocytes

That the HSPG is involved in the binding of ATF is suggested from studies in which keratinocytes were treated with heparitinase, chondroitinase ABC and phospholipase C prior to immunostaining with anti-ATF antibody. Treatment of the keratinocytes with heparitinase, but not chondroitinase nor phospholipase C, dramatically diminished the punctate staining for ATF compared to the control (data not shown), indicating involvement of HSPG in ATF binding to the cell surface.

To assess the physiological state of uPA in keratinocyte stimulation, the effect of GFD peptide was assayed by measuring thymidine incorporation by keratinocytes in the presence of 10 mg/ml of blocking antibody to uPA (#394), or anti-ATF antibody (#3471). In some of the experiments, keratinocytes cultures were supplemented with 0.3 unit/ml of heparitinase (EC 4.2.2.8) (Seikagaku, Rockville, MD) , 0.3 unit/ml of chondroitinase ABC (EC 4.2.2.4) (Seikagaku),or 1 unit/ml of phospholipase C (Seikagaku) overnight. The concentration of ^{3}H-thymidine was 10 mCi/ml. Cells were lysed, fixed with 70% ethanol and blotted onto a membrane filter using a Tomtec Mach II 96 harvester (Wallac Inc., Gaitherburg, MD). Incorporated radioactivity was measured using a 1205 Betaplate Liquid Scintilation Counter (Wallac Inc., Gaitherburg, MD).

10. Effect of the GFD-21 peptides on the generation of ATF

The generation of ATF by autolysis of uPA is a relatively slow process. Using anti-ATF antibody as a probe, ATF could be detected by Western blot analysis after 2 h of incubation of uPA. In addition to the 18kDa band of ATF, bands of 110, 53 and 35 kDa were also detected with the antibody. The 53kDa band apparently represents intact uPA and a faint 110 kDa band may be a dimer. The 35kDa band was not characterized but his band also seemed to be derived from uPA by degradation.

The generation of ATF was greatly accelerated in the presence of GFD-21 peptide. Ten minutes of incubation were sufficient to generate considerable amount of ATF. Prolonged incubation resulted in the degradation of ATF. At 2 h of incubation, ATF was no longer detectable. Only smaller degradation products were detectable. This is in sharp contrast to the control incubations in which a discrete ATF band was maintained.
Since heparatinase abolished the proliferative effect of GDF-21, we tested the effect of heparin on the generation of ATF. When uPA was incubated for 2 hours in the presence of heparin, the generation of ATF was completely suppressed. The generation of ATF was also

significantly reduced when heparin was added to incubation which included the GFD-21 peptide. Thus, the GFD-21 peptide can enhance the catalytic activity of uPA and generate ATF more rapidly. Furthermore, the autolysis can be inhibited by heparin. These results suggest that the mitogenic effect of the GFD-21 peptide may result from the generation of ATF and not from a direct interaction of the peptide with a cell surface molecule.

Two samples of purified uPA (5 mg each) were incubated for periods ranging from 10 min to 2 hrs. One of the samples was supplemented with 10 mM of GFD peptide prior to incubation. At appropriate intervals, samples were mixed with SDS sample buffer and heated at 95° C for 5 min. After SDS PAGE the separated proteins were transferred to a PVDF membrane (Amersham, Arlington Heights, IL). ATF generation was evaluated by Western blot analysis using anti-ATF antibody as a probe. Similar experiments were performed in the presence of 10 mg/ml of heparin.

11. Identification of ATF-syndecan complex formation

a) Solid phase binding assay with syndecans

Syndecan samples purified by immunoprecipitation (approx. 15,000 cpm/25 ml) from ^{35}S-sulfate labeled keratinocytes were allowed to interact with various amount of ATF, LMW-uPA and whole uPA that had been immobilized on a nitrocellulose membrane. BSA was used as a negative control. Only ATF bound syndecan in a dose dependent manner (Fig. 12). LMW-uPA which lacks the kringle domain and the EGF-like domain did not show any binding nor did uPA even with 50 mg of the protein.

b) Column chromatographic analysis

Approximately 0.8 ml of each ligand-bound Sepharose was packed in a minicolumn and the affinity for radiolabeled syndecan was analyzed under various conditions. When the syndecan sample was applied to a rATF-Sepharose column, 20 to 26 percent of the total radioactivity was absorbed to the column and eluted with 1 M NaCl (Fig. 13). Binding to a rATF column was completely blocked when the syndecan sample was mixed with 50 mg/ml of either heparin or chondroitin sulfate B, indicating ionic interaction between syndecan and ATF. The uPA-Sepharose column, in contrast, bound less than 5% of the labeled syndecan. These results are consistent with the results from the solid phase binding assay which indicated that ATF but not uPA is critical for the binding to syndecan.

Recombinant ATF (amino acid residues 1-135 of uPA) was constructed as a glutathione S-transferase (GST) fusion protein. ATF cDNA was obtained after RT-PCR using primers containing Bam HI starting site (upper primer: 5'AAAGGATCCAATGAACTTCATCAAGTTCCA3' (SEQ ID NO:29)) and Eco RI site in the end (lower primer: 5'AAAGAATTCCTTAAAGCGGGGCCTCAGAGT3' (SEQ ID NO:30)). The PCR product was ligated into the pGEX-2T vector (Pharmacia, Uppsala, Sweden) and was expressed in frame with GST. rATF-GST fusion protein was purified from bacterial lysates using glutathione-Sepharose 4B (Pharmacia, Uppsala, Sweden) affinity

chromatography. Immuno-reactivity of the fusion protein was tested by Western blot analysis using anti-ATF mAb #3471.

Cell surface heparan sulfate proteoglycan (syndecan) was prepared according to the method of Baciu et al. (1994) *J. Biol. Chem.* 269: 696-703) using the polyclonal antibody MS-1-C directed against the cytoplasmic domain of mouse syndecan-1. The cytoplasmic domain of syndecan is highly conserved among all the family members and shown to react with all types of syndecans as well as syndecans in other species. For example, cytoplasmic domain of human and murine syndecans are 100% identical (Mali et al. (1990) *J. Biol. Chem.* 265: 6884-6889). Briefly, keratinocytes were metabolically labeled with 100 mCi/ml of [^{35}S] H_2SO_4 overnight. Lysis buffer (PBS containing 1% Nonidet P-40, 1 mM EDTA, 1 mM EGTA, 0.1 mg/ml of aprotinin, 0.2 mg/ml of [4-(2-aminoethyl)-benzensulfonyl fluoride/HCl] (CalBiochem, San Diego, CA), 10 mg/ml of leupeptin and 10 mg/ml of pepstatin) was added to the culture dish and kept on ice for 20 min. After scraping, the lysates were centrifuged for 30 min at 4° C. The supernatant was mixed with 50 ml of protein-G beads (Pierce Chemical Co., Rockford, IL) previously reacted with MS-1-C. Immunoprecipitation was performed for 1 h at room temperature. Beads were then extensively washed four times with lysis buffer containing 0.5 M NaCl and resuspended in PBS. For binding assays, syndecan was released from the beads using 0.1 M glycine (pH 2.5) and the pH was adjusted to 7.0 with 1 M Tris-HCl (pH 8.0).

The experiments described above characterized the mitogenic property of uPA. It has been shown herein that uPA growth mechanism involves cell surface heparan sulfate proteoglycan, also known as syndecan.

The studies described herein revealed that the regulation of the production and secretion of uPA was dependent on both the cell cycle and the stage of the culture. Only the proliferating keratinocytes produced uPA. Northern blot analysis clearly showed that steady state levels of uPA mRNA were found only in proliferating cells and not when keratinocytes reached confluency. Grimaldi et al. (1986) *EMBO J.* 5:855-861) reported that mouse keratinocytes growth stimulation was correlated with the induction of uPA mRNA. They also showed a decrease in uPA expression in differentiated keratinocytes. Since keratinocyte differentiation is correlated with cessation of proliferation, these results suggest a relationship between uPA expression and cell proliferation.

The observation that ATF can stimulate thymidine incorporation by keratinocytes demonstrates that this portion of uPA possesses mitogenic properties. Thus, ATF may be a mitogenic factor for several different cell types. A synthetic peptide from the EGF-like domain (uPA12-32) was also used to define the minimum domain structure for growth stimulation, since that domain had been shown to compete with uPA for binding to uPAR (Appella et al. (1987) *J. Biol. Chem.* 262:4437-4440). Continuous exposure of keratinocytes to 1 mM GFD peptide was found to cause a 50% increase in cell number after nine days of culture compared to the control. Although the EGF-like domain is the binding site of uPA to

uPAR, uPAR is not believed to be involved in the growth signal transduction for three reasons. First, uPAR is a GPI-anchored protein and its cytoplasmic domain doesn't possess any consensus sequence for tyrosine kinases (Roldan et al. (1990) *EMBO J.* 9:467-474). The cytoplasmic domain is composed of only ten amino acids which are mostly hydrophobic. This indicates that even if the cytoplasmic domain remains intact, it will be buried in the membrane and will not be available for association with other tyrosine kinases. Second, kinetic analysis of uPA-uPAR interaction demonstrated the existence of high and low affinity uPA binding receptors in A431 cells (Fibbi et al. (1986), *Biochim. Biophys. Acta* 885:301-308) suggesting that the uPAR may not be the only receptor for uPA. And third, the immunohistochemical results reveal the presence of a uPA binding substance which is clearly distinct from uPAR. Hence, uPAR is not the surface molecule associated with the growth stimulatory activity of uPA.

This molecule has been identified as the cell surface HSPG of the syndecan family. Syndecan is known as a low affinity receptor for heparin-binding growth factors such as FGFs (Kim et al. (1994) *Mol. Biol. Cell.* 5:797-805). Also, syndecan is essential for high affinity binding of tyrosine kinase receptors (Rapraeger et al. (1991) *Science* 252:1705-1708) and Yayon et al. (1991) *Cell.* 64:841-848). In solid phase membrane assay and affinity chromatographic analysis it was shown that only ATF can bind syndecans. uPA, the parent molecule, which possesses ATF in its structure failed to bind syndecans. It has been assumed that the kringle domain of ATF is responsible for the binding to syndecans. This could be interpreted as follows. Once ATF is released from uPA, conformational changes occur, resulting in the exposure of heparan sulfate binding sequence. In the kringle domain of uPA, there is a cluster of positively charged amino acids between Cys102 and Cys 113 (Blasi et al. (1986) in F. Blasi (Ed). *Human genes and diseases*, John Wiley & Sons, Ltd., 377-414). This part (CRNPDNRRRPWC (SEQ ID NO:31)) fits quite nicely into the heparin binding consensus sequence, XBBBXXXBX, where B is a basic amino acid (A.D. Cardin and H.J.R. Weintraub (1989) *Arteriscler* 9:21-32).

The fact that growth stimulation is limited to ATF implies elaborate and yet indispensable feature of this mechanism, given that uPA is produced by various kind of cell types and relatively abundant in many tissues. The generation of ATF, however, depends on the catalytic activity of uPA. The blocking antibody against uPA suppressed thymidine incorporation, whereas anti-ATF antibody did not. The observed mitogenic activity of uPA may reflect the complicated feature of this mechanism which includes generation of ATF and the availability of syndecans.

Four members of the syndecan family have been identified to date (K. Elenius and M. Jalkanen, (1994) *J. Cell Sci.* 107:2975-2982). All members have glycosaminoglycan chains attached to the ectodomain. The cytoplasmic domains of syndecans are highly conserved not only in the all four types of syndecans but also among different species. However, there are distinct subdomains which are specific to the family members. For example, syndecan-4

contains a focal contact localization signal as predicted by its presence within focal contacts (Baciu et al. (1994) *J. Biol. Chem* 269:696-703 and A. Woods and J.R. Couchman (1994) *Mol. Biol. Cell* 5:183-192). On the other hand, syndecan-1 lacks this sequence and is not found within focal contacts (Carey et al. (1994) *J. Cell Biol.* 124: 161-170). An antibody directed against the cytoplasmic domain of mouse syndecan-1 was used for the immunoprecipitation of the human syndecan fraction. In the affinity chromatographic analysis, only 20-26% of the labeled syndecan fraction was bound to the recombinant ATF column. This was not due to the column capacity, since diluted syndecan samples showed the similarly proportional binding (20-25%). Sanderson et al. ((1992) *J. Invest. Dermatol.* 99:3990-396) reported localization of syndecan-1 in the epidermis. The results suggest that keratinocytes may express more than one species of syndecan and that only a particular type of syndecan can bind ATF. Multiple types of syndecans are commonly expressed in many cell types (Kim et al. (1994) *Mol. Biol. Cell* 5:797-805).

Requirement of syndecan is also critical for the signal transduction by the GFD peptide. If heparin is present in the medium, thymidine incorporation by GFD peptide was completely blocked. Treatment with heparitinase abolished growth stimulation by the peptide, whereas chondroitinase ABC or GPI-specific phospholipase C did not affect thymidine incorporation. *In vitro* experiments revealed that the GFD peptide strongly affect the generation of ATF. The GFD peptide seems to work as an enhancer of uPA catalytic activity, liberating ATF in a short time period, though it also causes degradation of ATF very quickly. From these results, it has been concluded that the GFD peptide works through the generation of ATF. Interestingly, heparin protected this autolysis process. Similar results were reported by Saksela et al. (1988) *J. Cell Biol.* 107:743-751 who showed the protective effect of endothelial cell-derived heparan sulfate on basic FGF from plasmin digestion. Apparently, heparin or heparan sulfate proteoglycan has a protective function against proteolytic degradation. This property of heparan sulfate is quite beneficial for growth stimulation by ATF. Since uPA doesn't bind to the syndecans, generation of ATF by autolysis would not be affected even if this phenomenon occurs near syndecans. Once ATF is generated and binds to a syndecan, it will be protected from further digestion caused by uPA or plasmin.

A model for the mechanism of growth stimulation by uPA is illustrated in Fig. 14. uPA generates ATF under certain circumstances through autolysis process. The GFD peptide can accelerate the production of ATF by a yet unknown mechanism. This may occur at sites of wound healing or cancer cell metastasis where proteolytic degradation plays an important role. The GFD domain could be proteolytically released facilitating the generation of ATF. Liberated ATF would then undergo a conformational change and heparan sulfate binding sequence would be exposed. The last step outside the cell would be the binding of ATF to a particular type of syndecan on the cell surface, transmitting a growth signal through an associated protein with kinase activity. For the signal transduction by the ATF-syndecan

complex, it is possible to postulate the involvement of a kinase receptor as shown in the case of FGFs.

Growth stimulation by uPA may not be a commonly utilized mechanism in normal physiological condition, due to the following reasons. First, this growth stimulation definitely requires a high level of uPA production in order to generate ATF as seen in cultured keratinocytes and some cancer cells. The results showed that uPA activity in the medium was detectable throughout the keratinocyte culture, indicating that the level of uPA production is exceeded from that of uPAR, thus uPAR in the keratinocytes would be constitutively saturated. This implies that the free form of uPA is always available in the culture and can be used as the source of ATF. These circumstances may explain why uPA can act as a mitogen in cultured keratinocytes and in malignant cells. Second, the availability of syndecan further restrict its role, therefore, the uPA-ATF-syndecan system appears to be different from ordinary growth stimulation. This mechanism is certainly related to unusual cell growth not only in cancer cell proliferation but also in wound healing process, where the keratinocytes around the damaged area express high levels of uPA and respond to repair by extensive cell proliferation (Grønhahl-Hansen et al. (1988) *J. Invest. Dermatol.* 90:790-795 and Rømer et al. (1991) *J. Invest. Dermatol.* 97:803-811).

Use

The formulations of this invention are especially useful for topical administration, but may also be administered in other modes, e.g., parenterally, intravenously, subcutaneously, or intramuscularly. Therapeutically effective amounts (e.g., amounts which eliminate or reduce the patient's pathological condition) of the peptides of the invention can be administered to humans or other mammals to treat or inhibit conditions or disorders wherein inhibition or stimulation of cell growth is desired, e.g., in disorders wherein the promotion of keratinocyte growth is desirable.

The compounds provided herein can be formulated into pharmaceutical compositions by admixture with any pharmaceutically acceptable nontoxic excipients and carriers. As noted above, such compositions may be prepared for parenteral administration, particularly in the form of liquid solutions or suspensions; for oral administration, particularly in the form of tablets or capsules; or topically in the form of ointments, creams or gels. The compositions may conveniently be administered in unit dosage form and may be prepared by any of the methods well known in the pharmaceutical art. Formulations for parenteral administration may contain as common excipients, sterile water or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes and the like. In particular, biocompatible, biodegradable lactide polymer, lactide/glycoside copolymer, or polyoxyethylene-polyoxypropylene copolymers may be useful excipients to control the release of the peptides. Other potentially useful parenteral delivery systems for these peptides include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for parenteral administrations may also

include glycocholate for buccal administration, methoxysalicylate for rectal administration, or citric acid for vaginal administration.

The materials of this invention can be employed as the sole active agent in a pharmaceutical composition or can be used in combination with other active ingredients, e.g., other compounds which facilitate cell growth or inhibition, or peptidase or protease inhibitors.

The dosage of the compounds described herein in a therapeutic composition will vary depending upon a number of factors, including the route of administration, type and state of the disease, and the overall health status of the particular patient.

The peptides of the invention can also be used in vitro, e.g., to stimulate the growth of cultured epidermal cells.

Other Embodiments

The invention includes any peptide which is substantially homologous to a uPA peptide described herein and which has biological activity. By "biologically active" is meant the ability to bind specifically to a uPAR and/or a cell surface heparan sulafte proteoglycan, e.g., a syndecan, e.g., a syndecan-1, 2, 3 or 4, or any combination thereof, bearing cell, e.g., a keratinocyte, the ability to promote or inhibit the growth (mitogenesis) of a uPAR and/or a cell surface heparan sulafte proteoglycan, e.g., a syndecan, e.g., a syndecan-1, 2, 3 or 4, or any combination thereof, bearing cell, e.g., a keratinocyte, or the ability to modulate, e.g., enhance or inhibit, the uPA catalytic activity, as determined by the assays described herein or by other assays know to those in the art. Most preferably substantially homologous peptides, fragments or analogs will have: 10%, preferably 40%, more preferably at least 90, 95, or 99%, of the activity of the 9-mer and 6-mer of Table 1 and A-GFD, in the case of growth promoting peptides; and 10%, preferably 40%, more preferably at least 90, 95, or 99%, of the inhibitory activity of R-GFD or E-GFD, in the case of inhibitory peptides. Peptides which bind but have no effect on mitogenesis have biological activity if they bind at least 10, preferably 40, or more preferably at least 90, 95, or 99%, as well as the peptide of SEQ ID NO:1. The invention also includes chimeric peptides that include uPA peptides described herein.

The invention also includes any biologically active fragment or analog of the uPA peptides described herein. Preferred analogs include peptides whose sequences differ from the wild-type sequence (i.e., the sequence of the homologous portion of naturally occurring uPA) only by conservative amino acid substitutions, preferably by only one, two, or three, substitutions, for example, substitution of one amino acid for another with similar characteristics (e.g., valine for glycine, arginine for lysine, etc.) or by one or more non-conservative amino acid substitutions, deletions, or insertions which do not abolish the polypeptide's biological activity. Table 2 lists a number of conservative amino acid substitutions.

TABLE 2

CONSERVATIVE AMINO ACID REPLACEMENTS

For Amino Acid	Code	Replace With
Alanine	A	D-Ala, Gly, Aib, β-Ala, Acp, L-Cys, D-Cys
Arginine	R	D-Arg, Lys, D-Lys, homo-Arg, D-homo-Arg,Met, Ile, D-Met, D-Ile, Orn, D-Orn
Asparagine	N	D-Asn, Asp, D-Asp, Glu, D-Glu, Gln, D-Gln
Aspartic Acid	D	D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln
Cysteine	C	D-Cys, S-Me-Cys, Met, D-Met, Thr, D-Thr
Glutamine	Q	D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp
Glutamic Acid	E	D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln
Glycine	G	Ala, D-Ala, Pro, D-Pro, Aib, β-Ala, Acp
Isoleucine	I	D-Ile, Val, D-Val, AdaA, AdaG, Leu, D-Leu, Met, D-Met
Leucine	L	D-Leu, Val, D-Val, AdaA, AdaG, Leu, D-Leu, Met, D-Met
Lysine	K	D-Lys, Arg, D-Arg, homo-Arg, D-homo-Arg, Met, D-Met, Ile, D-Ile, Orn, D-Orn
Methionine	M	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val
Phenylalanine	F	D-Phe, Tyr, D-Thr, L-Dopa, His, D-His, Trp, D-Trp, Trans-3,4, or 5-phenylproline, AdaA, AdaG, cis-3,4, or 5-phenylproline, Bpa, D-Bpa
Proline	P	D-Pro, L-l-thioazolidine-4-carboxylic acid, D-or L-1-oxazolidine-4-carboxylic acid (Kauer, U.S. Patent (4,511.390)
Serine	S	D-Ser, Thr, D-Thr, allo-Thr, Met, D-Met, Met(O), D-Met(O), L-Cys, D-Cys
Threonine	T	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Met(O), D-Met(O), Val, D-Val
Tyrosine	Y	D-Tyr, Phe, D-Phe, L-Dopa, His, D-His
Valine	V	D-Val, Leu, D-Leu, Ile, D-Ile, Met, D-Met, AdaA, AdaG

Other useful modifications include those which increase peptide stability; such analogs may contain, for example, one or more non-peptide bonds (which replace the peptide bonds) or D-amino acids in the peptide sequence.

Analogs can differ from naturally occurring uPA sequence in amino acid sequence or can be modified in ways that do not involve sequence, or both. Analogs of the invention will generally exhibit at least 40%, more preferably 50%, more preferably 60%, more preferably 70%, more preferably 80%, more preferably 90%, and most preferably 95% or even 99%, homology with a naturally occurring uPA sequence or with a uPA sequence described herein.

Non-sequence modifications include in vivo or in vitro chemical derivatization of peptides, e.g., acetylation, methylation, phosphorylation, carboxylation, or glycosylation.

Also included are analogs that include residues other than naturally occurring L-amino acids, e.g., D-amino acids or non-naturally occurring or synthetic amino acids, e.g., β or γ amino acids. Alternatively, increased stability may be conferred by cyclizing the peptide molecule.

Analogs, e.g., peptides differing by 1,2,3, or more residues from the peptides disclosed herein, can be prepared by methods known to those in the art and tested for biological activity by methods known in the art or disclosed herein.

Other embodiments are within the following claims.

What is claimed is:

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: HIBINO, Tashihiko; TAKAHASHI, Tadahito; HORII, Izumi; GOETINCK, Paul F.; and BACIU, Peter

(ii) TITLE OF INVENTION: UROKINASE PLASMINOGEN ACTIVATOR FRAGMENTS

(iii) NUMBER OF SEQUENCES: 31

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: LAHIVE & COCKFIELD
(B) STREET: 60 State Street, Suite 510
(C) CITY: Boston
(D) STATE: Massachusetts
(E) COUNTRY: USA
(F) ZIP: 02109

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: ASCII Text

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/142,590
(B) FILING DATE: 25-OCT-1993

(viii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/042,318
(B) FILING DATE: 02-APR-1993

(ix) ATTORNEY/AGENT INFORMATION:
(A) NAME: Myers, Paul L.
(B) REGISTRATION NUMBER: 35,965
(C) REFERENCE/DOCKET NUMBER: MGP-009C2PC

(x) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (617) 227-7400
(B) TELEFAX: (617) 227-5941

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Ser Asn Lys Tyr Phe Ser Asn Ile His
1 5

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Ser Asn Lys Tyr Phe Ser
1 5

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Asp Cys Leu Asn Gly Gly Thr Cys Val Ser Asn Lys Tyr Phe Ser Asn
1 5 10 15

Ile His Trp Cys Asn
20

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Asn Gly Gly Thr Cys Val Ser Asn Lys Tyr Phe Ser Asn Ile His Trp
1 5 10 15

Cys Asn

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Thr Cys Val Ser Asn Lys Tyr Phe Ser Asn Ile His Trp Cys Asn
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Thr Cys Val Ser Asn Lys Tyr Phe Ser Asn Ile His
1 5 10

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGGGATTGCC GTGTGGAAGA 20

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGAATTCGAA GGTAGCCACA GCCACGGAG 29

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Asp Cys Leu Asn Gly Gly Thr Cys Val Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Cys Leu Asn Gly Gly Thr Cys Val Ser
1 5

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Leu Asn Gly Gly Thr Cys Val Ser
1 5

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Asn Gly Gly Thr Cys Val Ser
1 5

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Gly Gly Thr Cys Val Ser
1 5

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Gly Thr Cys Val Ser
1 5

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Thr Cys Val Ser
1

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Ser Asn Ile His
1

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Ser Asn Ile His Trp
1 5

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Ser Asn Ile His Trp Cys
1 5

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Ser Asn Ile His Trp Cys Asn
1 5

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Ser Asn Ala Tyr Phe Ser Asp Ile His
1 5

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Ser Asn Arg Tyr Phe Ser Asp Ile His
1 5

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Ser Asn Glu Tyr Phe Ser Asp Ile His
1 5

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Ser Asn Ala Tyr Phe Ser
1 5

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Ser Asn Arg Tyr Phe Ser
1 5

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Ser Asn Glu Tyr Phe Ser
1 5

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GGGNATTGCC GTGTGGAAGA 20

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GGAATTCGAA GGTAGCCACA GCCACGGAG 29

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Asp Cys Leu Asn Gly Gly Thr Ala Val Ser Asn Lys Tyr Phe Ser Asn
1 5 10 15

Ile His Trp Cys Asn
20

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

AAAGGATCCA ATGAACTTCA TCAAGTTCCA 30

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

AAAGAATTCC TTAAAGCGGG GCCTCAGAGT 30

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Cys Arg Asn Pro Asp Asn Arg Arg Arg Pro Trp Cys
1 5 10

1. A purified uPA peptide in which the amino acid residue at position 23 is an amino acid other than L-lysine.

2. The purified peptide of claim 1, wherein the amino acid residue at position 23 is an amino acid having a side chain which is neutral at pH 7.

3. The purified peptide of claim 1, wherein the amino acid residue at position 23 is any of alanine, cysteine or glycine, or a neutral or neutral non-naturally occurring amino acid.

4. The purified uPA peptide of claim 1, wherein the amino acid residue at position 23 is an amino acid having a side chain with a net-positive charge at pH 7.

5. The purified uPA peptide of claim 1, wherein the amino acid residue at position 23 is an amino acid having a side chain which is more positively charged than is the side chain of lysine.

6. The purified uPA peptide of claim 1, wherein the amino acid residue at position 23 is a basic amino acid.

7. The purified uPA peptide of claim 1, wherein said amino acid residue at position 23 is any of arginine, histidine, or a positively charged or basic non-naturally occurring amino acid.

8. The purified uPA peptide of claim 1, wherein the amino acid residue at position 23 is an amino acid having a side chain with a net-negative charge at pH 7.

9. The purified uPA peptide of claim 1, wherein the amino acid residue at position 23 is an amino acid having a side chain which is more negatively charged than is the side chain of lysine.

10. The purified uPA peptide of claim 1, wherein the amino acid residue at position 23 is an acidic amino acid.

11. The purified uPA peptide of claim 1, wherein said amino acid residue at position 23 is any of aspartic acid, glutamic acid, or a negatively charged or acidic non-naturally occurring amino acid.

12. The purified uPA peptide of claim 1, wherein said peptide is a full length uPA peptide.

13. A purified uPA peptide comprising a sequence of the formula: n-R^2-Asn-R^1-Tyr-Phe-R^3-c, wherein,

R^1 is an amino acid residue other than L-lysine;

R^2 is a sequence of between 1 and 21 residues in length, having as its carboxy-terminus Ser^{21} of uPA and extending, inclusive of Ser^{21}, from between 1 and 21 amino acid residues in the N-terminal direction of uPA; and

R^3 is a sequence of between 1 and 25 residues in length having as its amino-terminus Ser^{26} of uPA and extending, inclusive of Ser^{26}, from between 1 and 25 amino acid residues in the C-terminal direction of uPA;

wherein c indicates the carboxy terminal direction of the peptide and n indicates the amino terminal direction of the peptide.

14. The purified uPA peptide of claim 13, wherein R^1 is an amino acid having a side chain with a net-positive charge at pH 7.

15. The purified uPA peptide of claim 13, wherein R^1 is a basic amino acid.

16. The purified uPA peptide of claim 13, wherein R^1 is any of arginine, histidine, or a positively charged or basic non-naturally occurring amino acid.

17. The purified uPA peptide of claim 13, wherein R^1 is arginine.

18. The purified uPA peptide of claim 13, wherein R^2 is any of:

n-Asp-Cys-Leu-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:9);

n-Cys-Leu-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:10);

n-Leu-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:11;

n-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:12);

n-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:13);

n-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:14);

n-Thr-Cys-Val-Ser-c (SEQ ID NO:15);

n-Cys-Val-Ser-c;

n-Val-Ser-c; or

n-Ser-c

19. The purified uPA peptide of claim 13, wherein R^3 is any of:

n-Ser-c;

n-Ser-Asn-c;

n-Ser-Asn-Ile-c;

n-Ser-Asn-Ile-His-c (SEQ ID NO:16);

n-Ser-Asn-Ile-His-Trp-c (SEQ ID NO:17);

n-Ser-Asn-Ile-His-Trp-Cys-c (SEQ ID NO:18); or

n-Ser-Asn-Ile-His-Trp-Cys-Asn-c (SEQ ID NO:19).

20. The purified uPA peptide of claim 13 wherein:

R^2 is n-Asp-Cys-Leu-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:9) and R^3 is n-Ser-Asn-Ile-His-Trp-Cys-Asn-c (SEQ ID NO:19);

R^2 is n-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:11);and R^3 is n-Ser-Asn-Ile-His-Trp-Cys-Asn-c (SEQ ID NO:19);

R^2 is n-Thr-Cys-Val-Ser-c (SEQ ID NO:15); and R^3 is n-Ser-Asn-Ile-His-Trp-Cys-Asn-c (SEQ ID NO:19);

R^2 is n-Thr-Cys-Val-Ser-c (SEQ ID NO:15); and R^3 is n-Ser-Asn-Ile-His-c (SEQ ID NO:16);

R^2 is n-Ser-c and R^3 is n-Ser-Asn-Ile-His-c (SEQ ID NO:16); or

R^2 is n-Ser-c and R^3 is n-Ser-c;

21. The purified peptide of claim 20, wherein said R^1 is Arg.

22. A purified uPA peptide comprising a sequence with the formula: n-R^2-Asn-R^1-Tyr-Phe-R^3-c, wherein,

R^1 is an amino acid residue other than L-lysine;

R^2 is a sequence of between 1 and 21 residues in length, having as its carboxy-terminus Ser^{21} of uPA and extending, inclusive of Ser^{21}, from between 1 and 21 amino acid residues in the N-terminal direction of uPA; and

R^3 is a sequence of between 1 and 25 residues in length having as its amino-terminus Ser^{26} of uPA and extending, inclusive of Ser^{26}, from between 1 and 25 amino acid residues in the C-terminal direction of uPA;

wherein c indicates the carboxy terminal direction of the peptide and n indicates the amino terminal direction of the peptide.

23. The purified uPA peptide of claim 22, wherein R^1 is an amino acid having a side chain with a net-negative charge at pH 7.

24. The purified uPA peptide of claim 22, wherein R^1 is an amino acid having a side chain which is more negatively charged than is the side chain of lysine.

25. The purified uPA peptide of claim 22, wherein R^1 is an acidic amino acid.

26. The purified uPA peptide of claim 22, wherein R^1 is any of aspartic acid, glutamic acid, or a negatively charged or acidic non-naturally occurring amino acid.

27. The purified uPA peptide of claim 26, wherein R^1 is glutamic acid.

28. The purified uPA peptide of claim 22, wherein R^2 is any of:

n-Asp-Cys-Leu-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:9);
n-Cys-Leu-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:10);
n-Leu-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:11);
n-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:12);
n-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:13);
n-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:14);
n-Thr-Cys-Val-Ser-c (SEQ ID NO:15);
n-Cys-Val-Ser-c;
n-Val-Ser-c; or
n-Ser-c

29. The purified uPA peptide of claim 22, wherein R^3 is any of:

n-Ser-c;
n-Ser-Asn-c;
n-Ser-Asn-Ile-c;
n-Ser-Asn-Ile-His-c (SEQ ID NO:16);
n-Ser-Asn-Ile-His-Trp-c (SEQ ID NO:17);
n-Ser-Asn-Ile-His-Trp-Cys-c (SEQ ID NO:18); or
n-Ser-Asn-Ile-His-Trp-Cys-Asn-c (SEQ ID NO:19).

30. The purified uPA peptide of claim 22 wherein:

R^2 is n-Asp-Cys-Leu-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:9) and R^3 is n-Ser-Asn-Ile-His-Trp-Cys-Asn-c (SEQ ID NO:19);

R^2 is n-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:11);and R^3 is n-Ser-Asn-Ile-His-Trp-Cys-Asn-c (SEQ ID NO:19);

R^2 is n-Thr-Cys-Val-Ser-c (SEQ ID NO:15); and R^3 is n-Ser-Asn-Ile-His-Trp-Cys-Asn-c (SEQ ID NO:19);

R^2 is n-Thr-Cys-Val-Ser-c (SEQ ID NO:15); and R^3 is n-Ser-Asn-Ile-His-c (SEQ ID NO:16);

R^2 is n-Ser-c and R^3 is n-Ser-Asn-Ile-His-c (SEQ ID NO:16); or

R^2 is n-Ser-c and R^3 is n-Ser-c;

31. The purified peptide of claim 30, wherein R^1 is glutamic acid.

32. A purified uPA peptide comprising a sequence of the formula: n-R^2-Asn-R^1-Tyr-Phe-R^3-c, wherein,

R^1 is an amino acid residue other than L-lysine;

R^2 is a sequence of between 1 and 21 residues in length, having as its carboxy-terminus Ser^{21} of uPA and extending, inclusive of Ser^{21}, from between 1 and 21 amino acid residues in the N-terminal direction of uPA; and

R^3 is a sequence of between 1 and 25 residues in length having as its amino-terminus Ser^{26} of uPA and extending, inclusive of Ser^{26}, from between 1 and 25 amino acid residues in the C-terminal direction of uPA;

wherein c indicates the carboxy terminal direction of the peptide and n indicates the amino terminal direction of the peptide.

33. The purified uPA peptide of claim 32, wherein R^1 is an amino acid having a side chain with a net-neutral charge at pH 7.

34. The purified uPA peptide of claim 32, wherein R^1 is a neutral amino acid.

35. The purified uPA peptide of claim 32, wherein R^1 is any of alanine, cysteine, glycine, or a neutral or neutral non-naturally occurring amino acid.

36. The purified uPA peptide of claim 32, wherein R^1 is alanine.

37. The purified uPA peptide of claim 32, wherein R^2 is any of:

n-Asp-Cys-Leu-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:9);

n-Cys-Leu-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:10);

n-Leu-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:11;

n-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:12);

n-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:13);

n-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:14);

n-Thr-Cys-Val-Ser-c (SEQ ID NO:15);

n-Cys-Val-Ser-c;

n-Val-Ser-c; or

n-Ser-c

38. The purified uPA peptide of claim 32, wherein R^3 is any of:

n-Ser-c;

n-Ser-Asn-c;

n-Ser-Asn-Ile-c;

n-Ser-Asn-Ile-His-c (SEQ ID NO:16);

n-Ser-Asn-Ile-His-Trp-c (SEQ ID NO:17);

n-Ser-Asn-Ile-His-Trp-Cys-c (SEQ ID NO:18); or

n-Ser-Asn-Ile-His-Trp-Cys-Asn-c (SEQ ID NO:19).

39. The purified uPA peptide of claim 32 wherein:

R^2 is n-Asp-Cys-Leu-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:9) and R^3 is n-Ser-Asn-Ile-His-Trp-Cys-Asn-c (SEQ ID NO:19);

R^2 is n-Asn-Gly-Gly-Thr-Cys-Val-Ser-c (SEQ ID NO:11);and R^3 is n-Ser-Asn-Ile-His-Trp-Cys-Asn-c (SEQ ID NO:19);

R^2 is n-Thr-Cys-Val-Ser-c (SEQ ID NO:15); and R^3 is n-Ser-Asn-Ile-His-Trp-Cys-Asn-c (SEQ ID NO:19);

R^2 is n-Thr-Cys-Val-Ser-c (SEQ ID NO:15); and R^3 is n-Ser-Asn-Ile-His-c (SEQ ID NO:16);

R^2 is n-Ser-c and R^3 is n-Ser-Asn-Ile-His-c (SEQ ID NO:16); or

R^2 is n-Ser-c and R^3 is n-Ser-c;

40. The purified peptide of claim 39, wherein said R^1 is Ala.

41. A therapeutic composition comprising as an active ingredient a peptide of the invention and a pharmaceutically-acceptable carrier.

42. A method of regulating growth of a cell comprising administering to said cell a growth regulating amount of a peptide of claim 1.

43. A method of regulating growth of epidermal tissue in a patient which has been subjected to trauma comprising administering to said tissue a growth regulating amount of a peptide of claim 1.

44. A method of regulating the growth of epidermal cells *in vitro* comprising culturing epidermal cells in the presence of a peptide of claim 1.

45. A method for treating an area of denuded skin in a patient comprising applying epidermal cells produced according to the method of claim 44 to allow effective attachment of said sheet to the underlying dermis.

46. A method of inhibiting the proteolytic destruction of an extracellular protein matrix which includes cells bearing the uPAR and cell surface heparin sulfate proteoglycans, comprising contacting cells bearing the uPAR with a peptide of claim 1.

47. A method of decreasing cell proliferation, comprising inhibiting the formation or activity of an ATF fragment of uPA.

48. A method of modulating uPA autolysis, comprising contacting a cell with an effective amount of an autolysis modulating substance.

49. A method for modulating the growth, healing, or adhesion attachment, of a cell bearing a cell surface heparin sulfate proteoglycan, comprising contacting said cell with an ATF, provided one or more of the following:

said cell is other than a keratinocyte;

said cell is other than an osteoblast;

said cell is other than a dermal cell;

said cell and said ATF are contacted in vivo.

50. A method for modulating the growth, healing, or adhesion attachment, of a cell bearing a cell surface heparin sulfate proteoglycan by inhibiting the binding of an ATF to a syndecan on said cell, provided one or more of the following:

said cell is other than a keratinocyte;

said cell is other than an osteoblast;

said cell is other than a dermal cell;

said cell and said ATF are contacted in vivo.

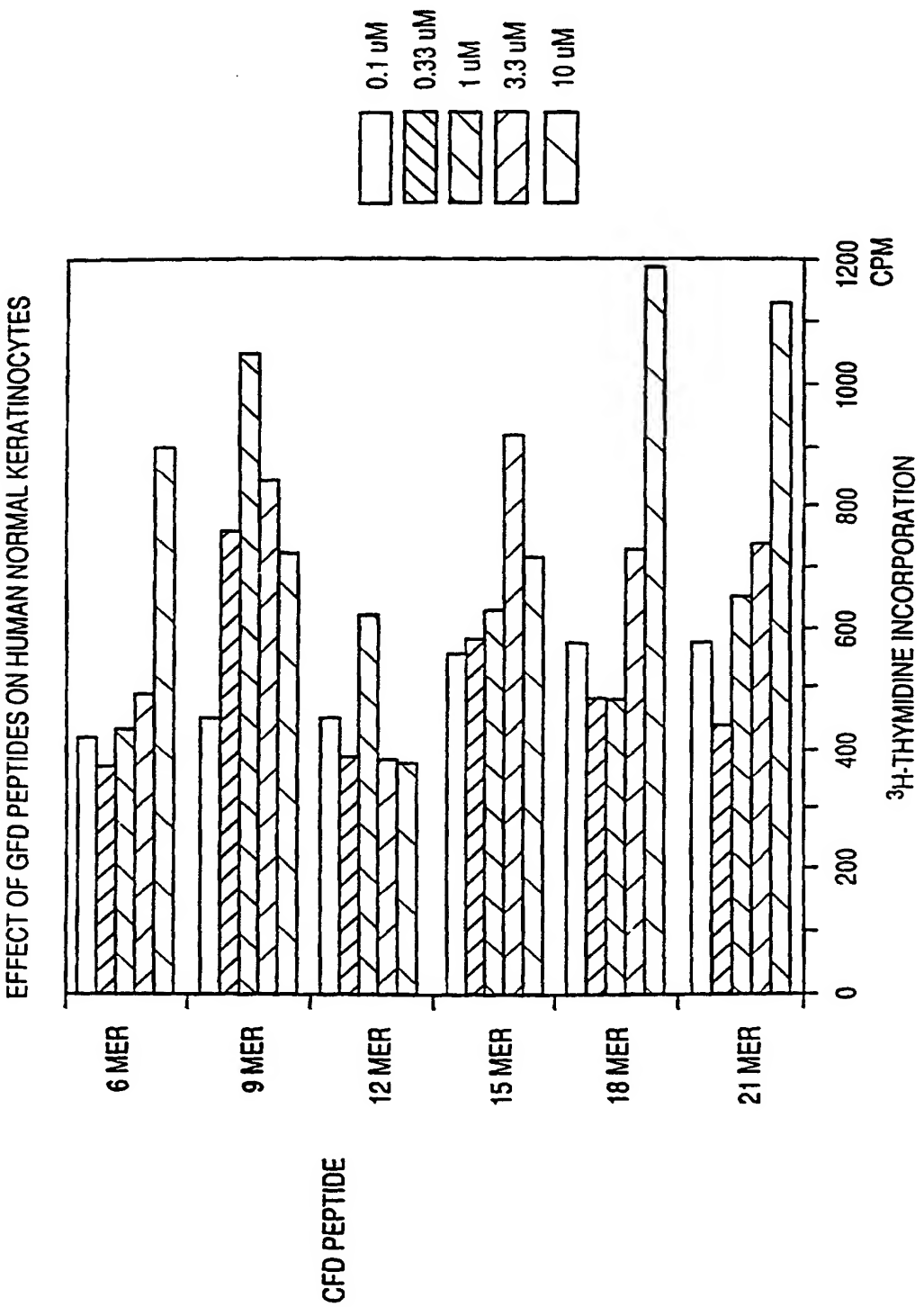
0.1 uM
0.33 uM
1 uM
3.3 uM
10 uM
EFFECT OF GFD PEPTIDES ON HUMAN NORMAL KERATINOCYTES
0
200
400
600
800
1000
1200
CPM
^{3}H-THYMIDINE INCORPORATION
6 MER
9 MER
12 MER
15 MER
18 MER
21 MER
CFD PEPTIDE

FIG. 1

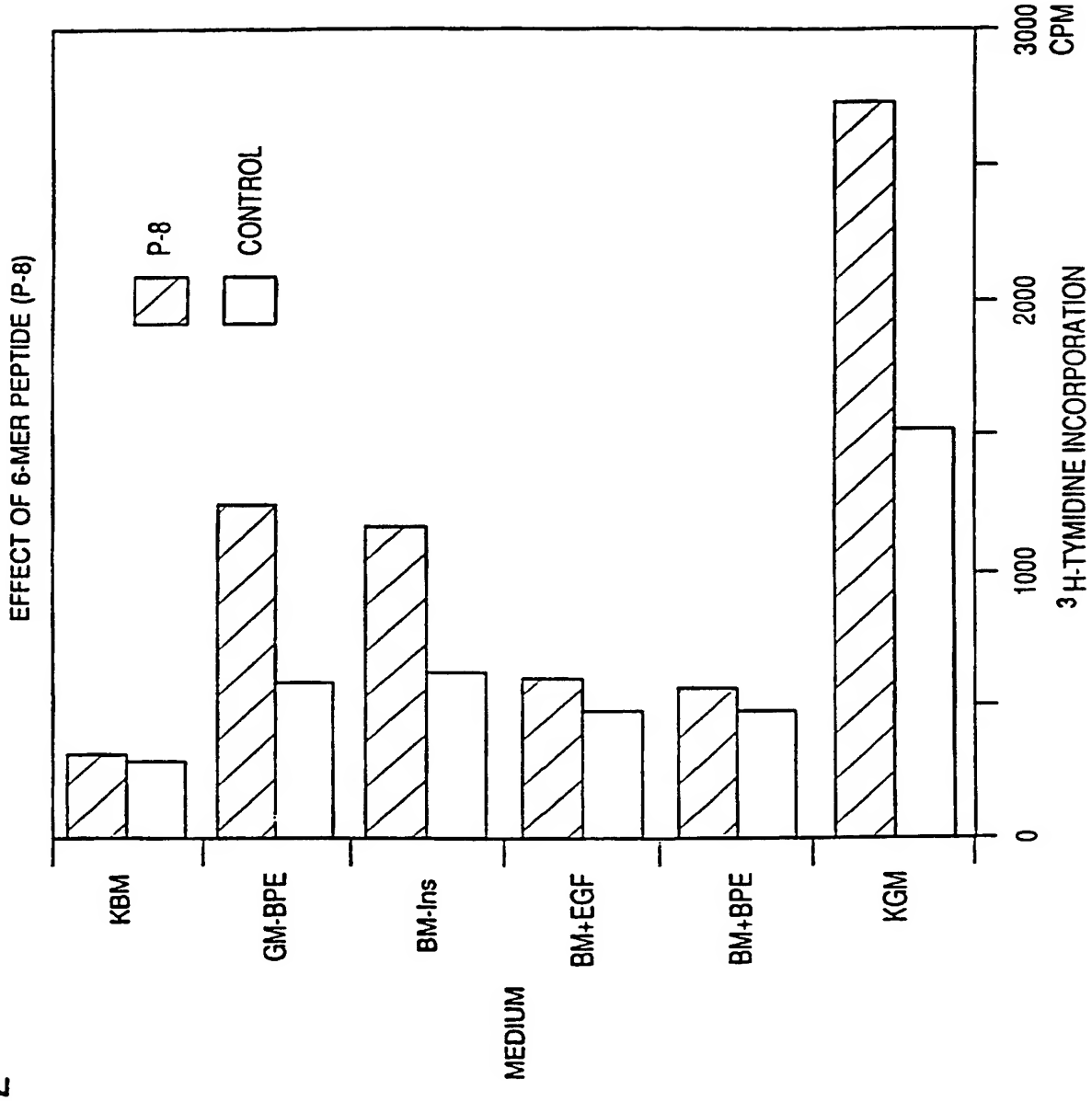
EFFECT OF 6-MER PEPTIDE (P-8)
P-8
CONTROL
3000
2000
1000
0
CPM
^{3}H-TYMIDINE INCORPORATION
KBM
GM-BPE
BM-Ins
BM+EGF
BM+BPE
KGM
MEDIUM

FIG. 2

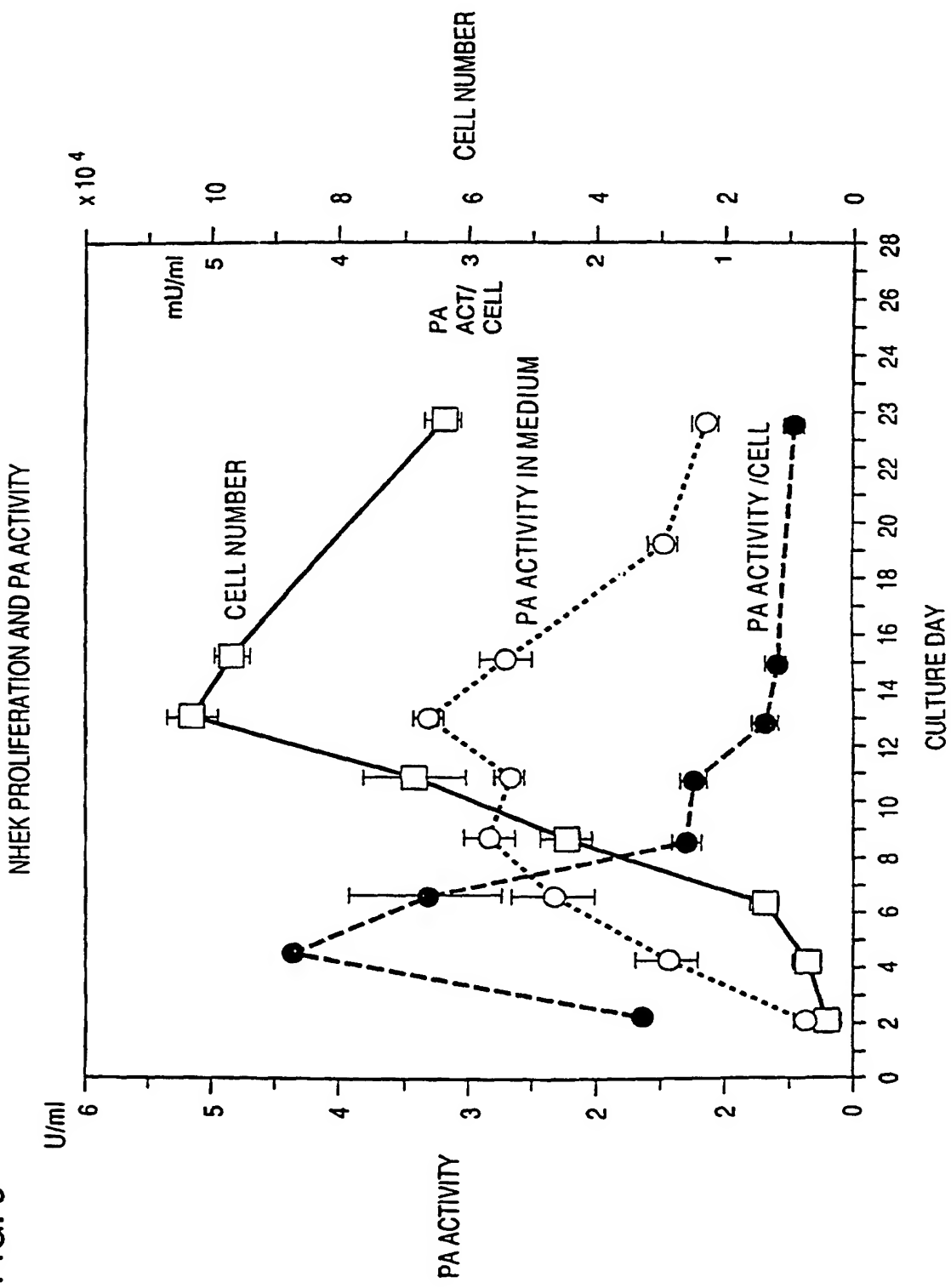
NHEK PROLIFERATION AND PA ACTIVITY
CELL NUMBER
PA ACTIVITY IN MEDIUM
PA ACTIVITY /CELL
U/ml
PA ACTIVITY
mU/ml
PA ACT/ CELL
x 10^4
CELL NUMBER
CULTURE DAY

FIG. 3

Synthetic GFD Peptides

Urokinase

```
1         10        20        30
SNELHQVPSNCDCLNGGTCVSNKYFSNIHWCNCPK
```

Synthetic Peptides

```
          12                    32
21 mer:  DCLNGGTCVSNKYFSNIHWCN
18 mer:     NGGTCVSNKYFSNIHWCN
15 mer:        TCVSNKYFSNIHWCN
12 mer:        TCVSNKYFSNIH
 9 mer:           SNKYFSNIH
 6 mer:           SNKYFS
```

FIGURE 4

Urokinase

1 10 20 30
SNELHQVPSNCDCLNGGTCVSNKYFSNIHWCNCPK

Synthetic GFD peptides

21 mers

12 32
GFD-21(C): DCLNGGTCVSNKYFSNIHWCN
GFD-21(A): DCLNGGTAVSNKYFSNIHWCN

9 mers with modifications

GFD-9K: SNKYFSNIH
GFD-9A: SNAYFSNIH
GFD-9R: SNRYFSNIH
GFD-9E: SNEYFSNIH

6 mers with modifications

GFD-6K: SNKYFS
GFD-6A: SNAYFS
GFD-6R: SNRYFS
GFD-6E: SNEYFS

FIGURE 5

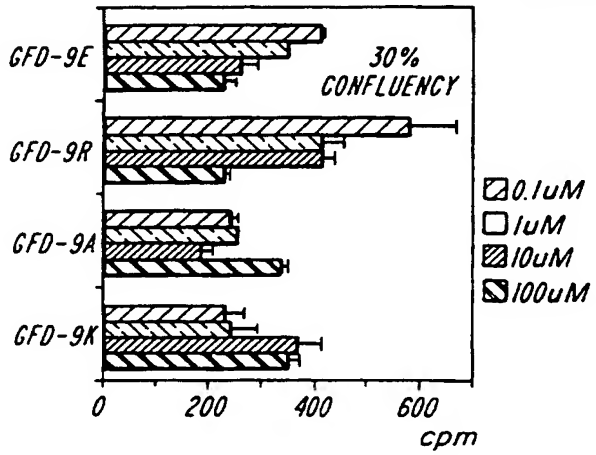

FIG. 6A

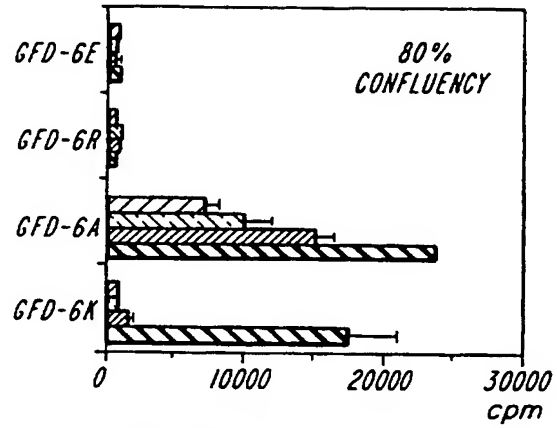

FIG. 6D

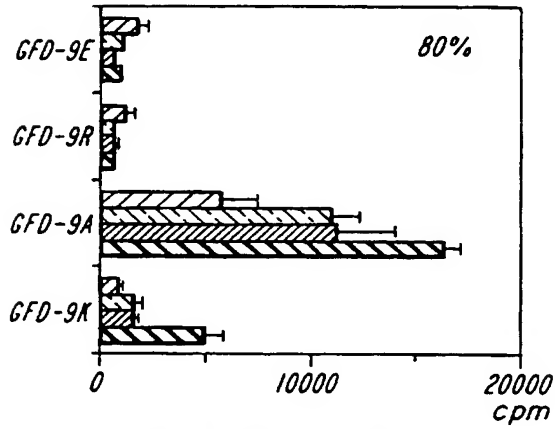

FIG. 6B

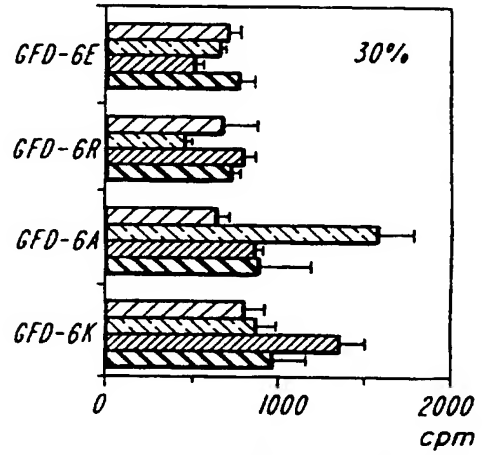

FIG. 6E

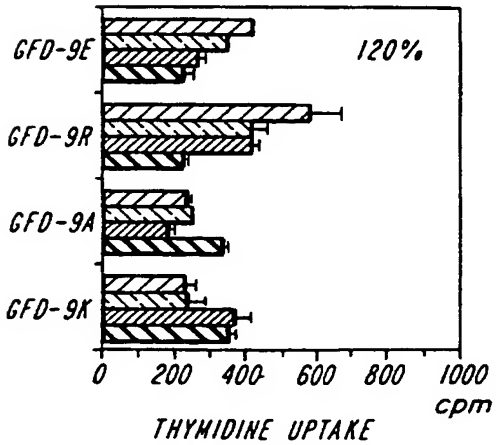

FIG. 6C

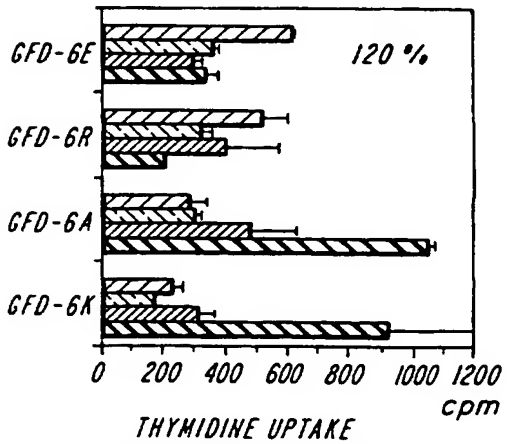

FIG. 6F

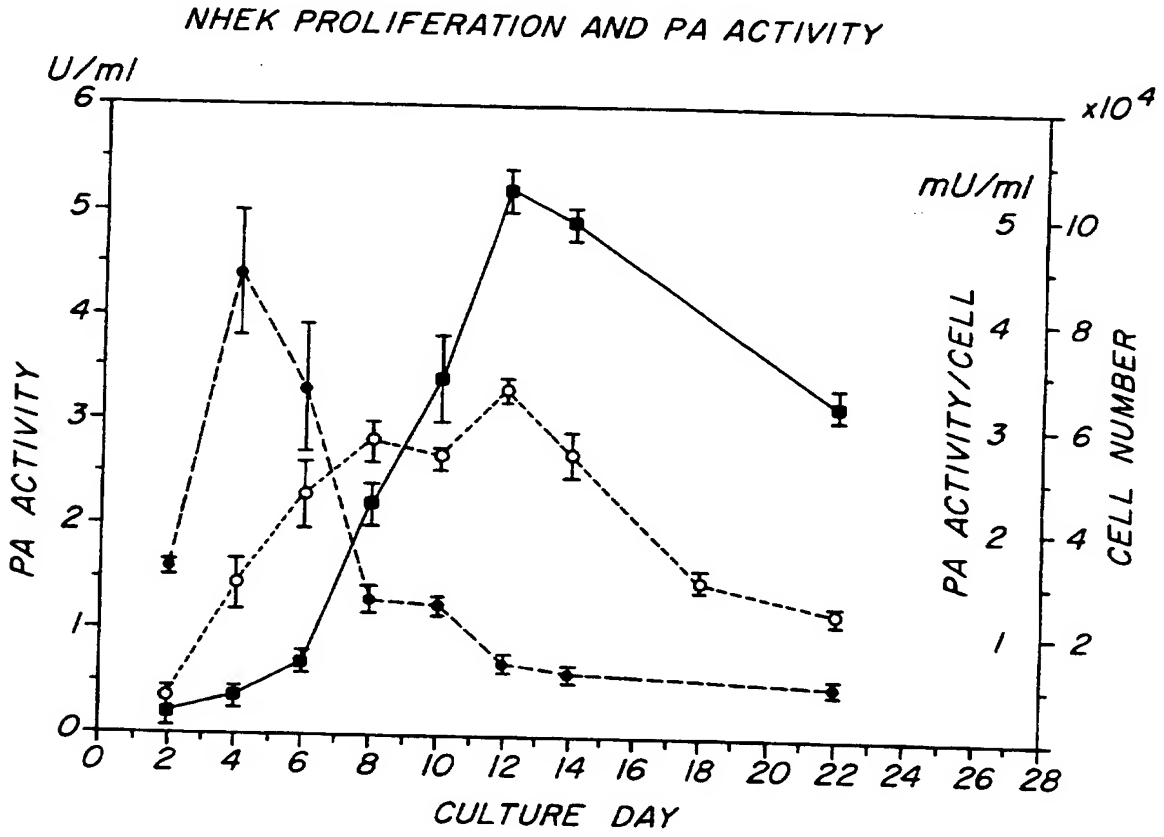

FIG. 7

CELL SYNCHRONIZATION AND PA ACTIVITY

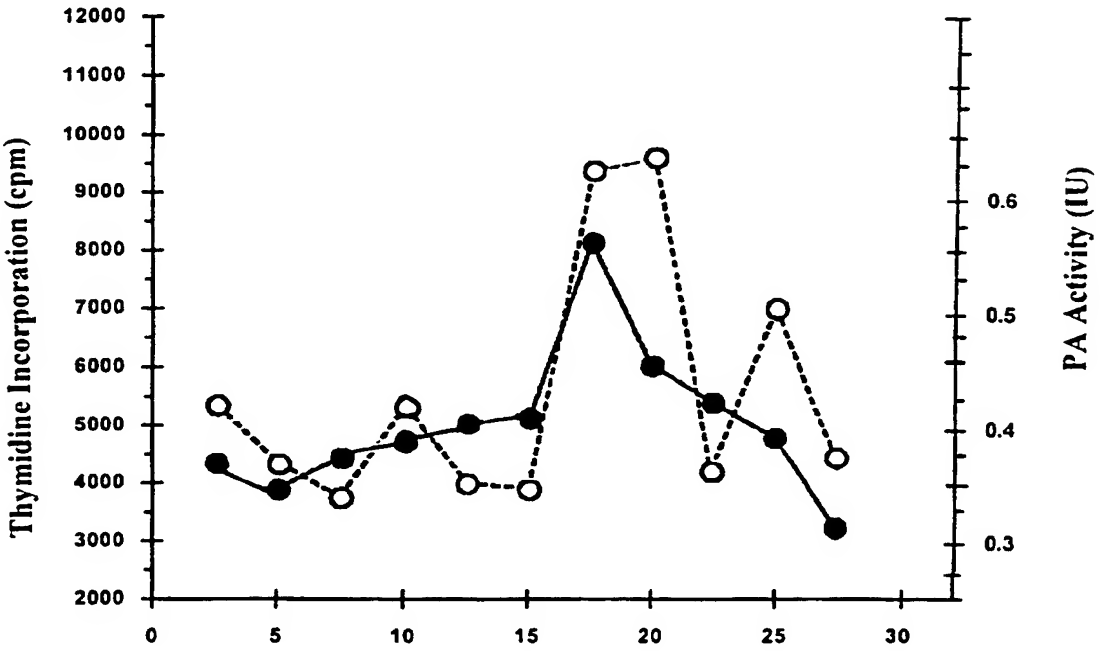

FIGURE 8

EFFECT OF ATF ON SYNCHRONIZED KERATINOCYTES

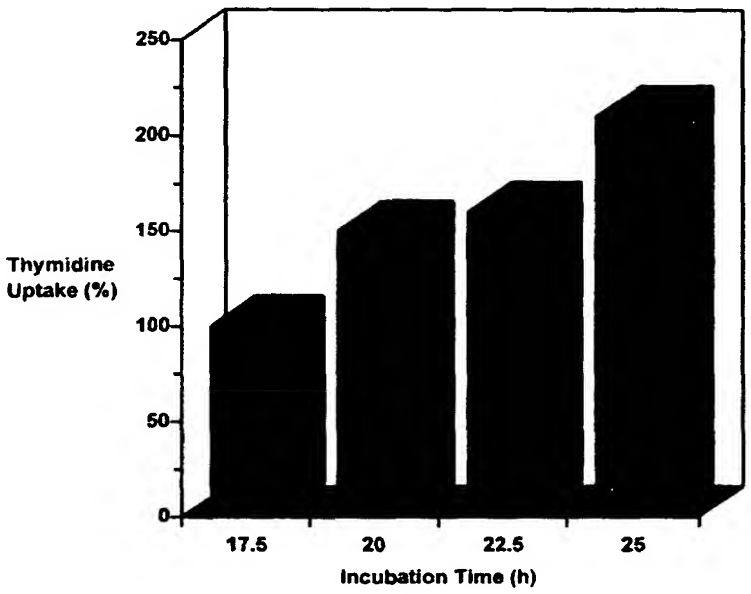

FIGURE 9

EFFECT OF GFD-21(A) ON KERATINOCYTE PROLIFERATION

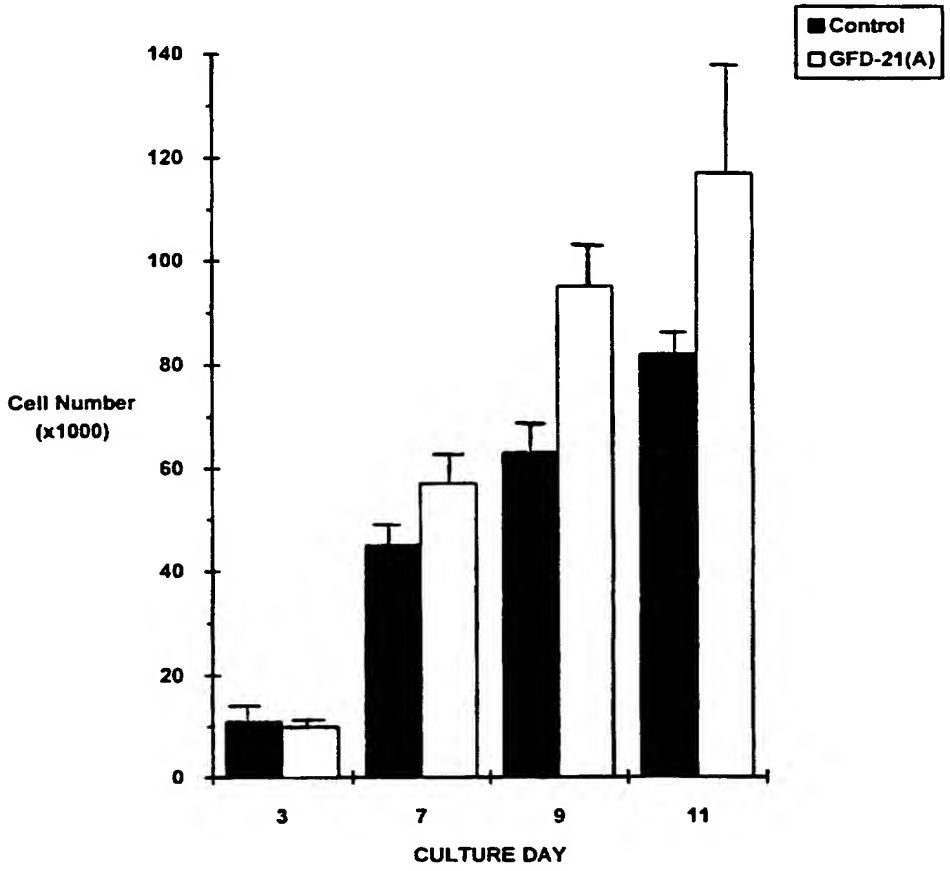

FIGURE 10

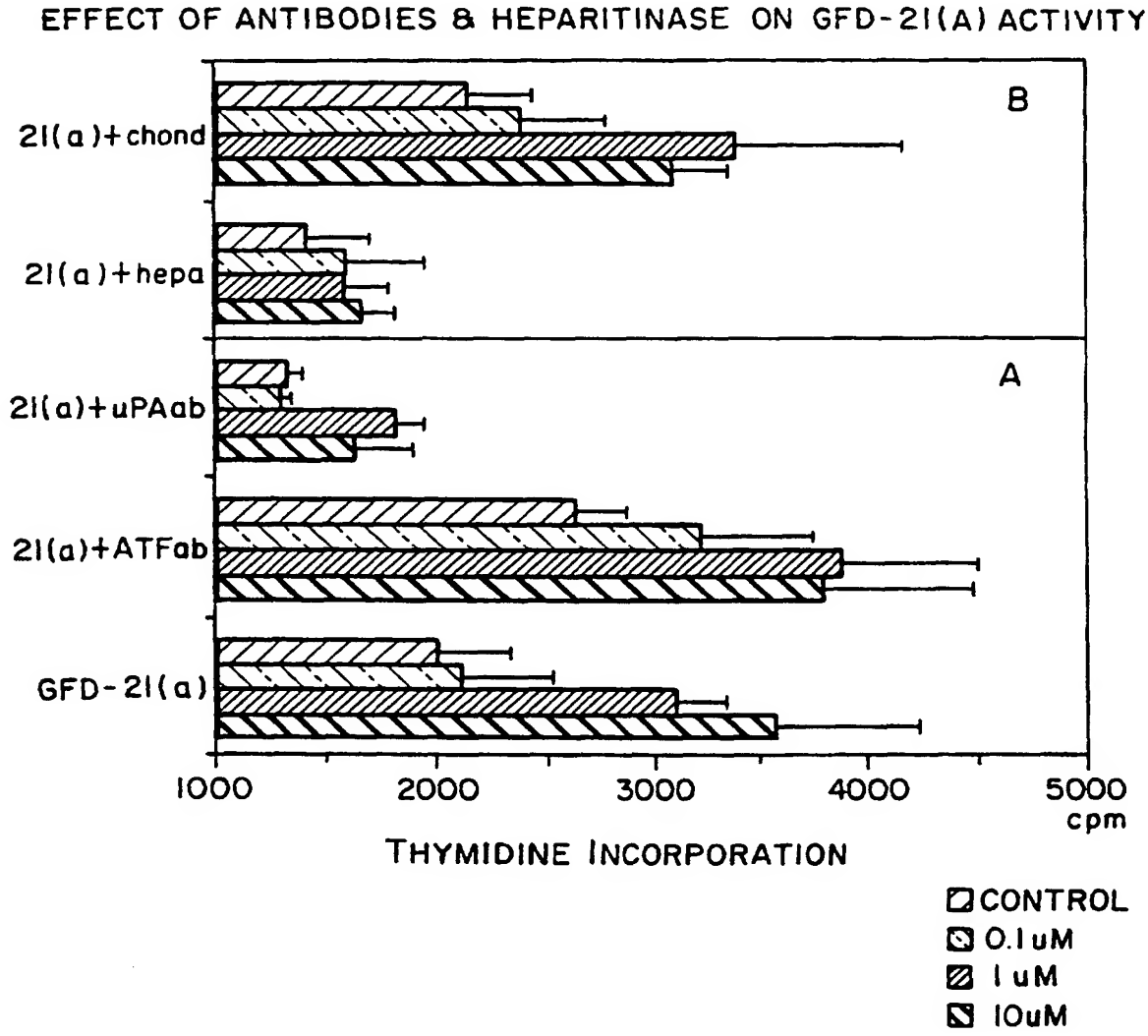

FIG. 11

BINDING OF SYNDECAN TO uPA

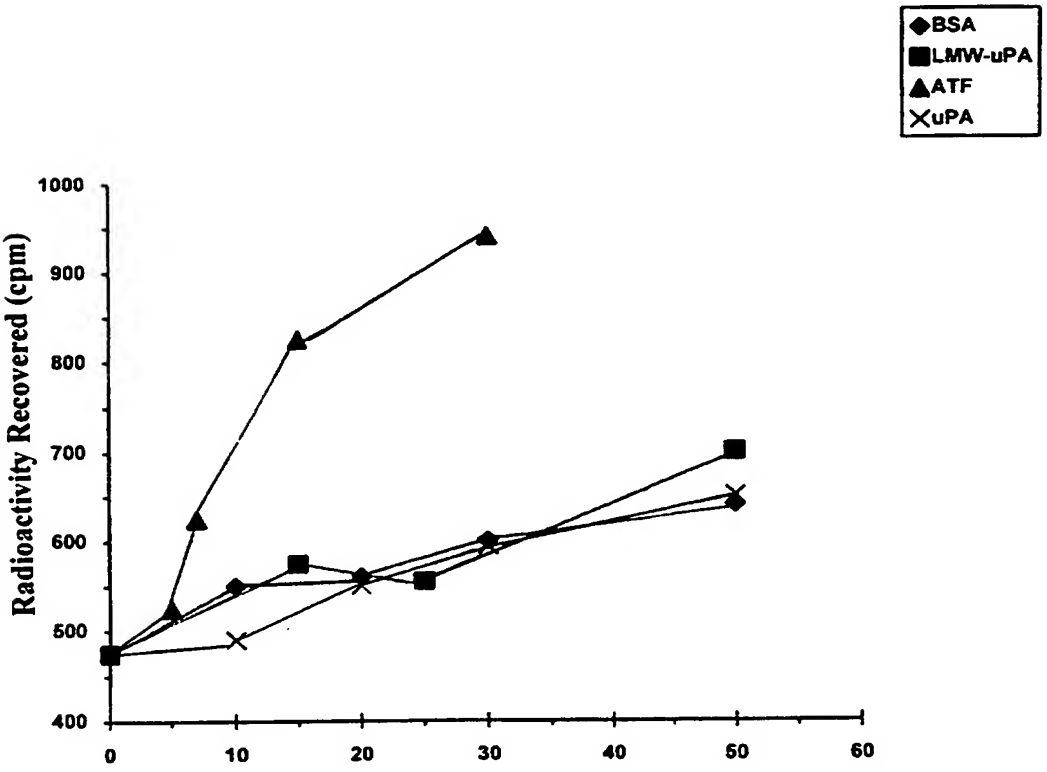

FIGURE 12

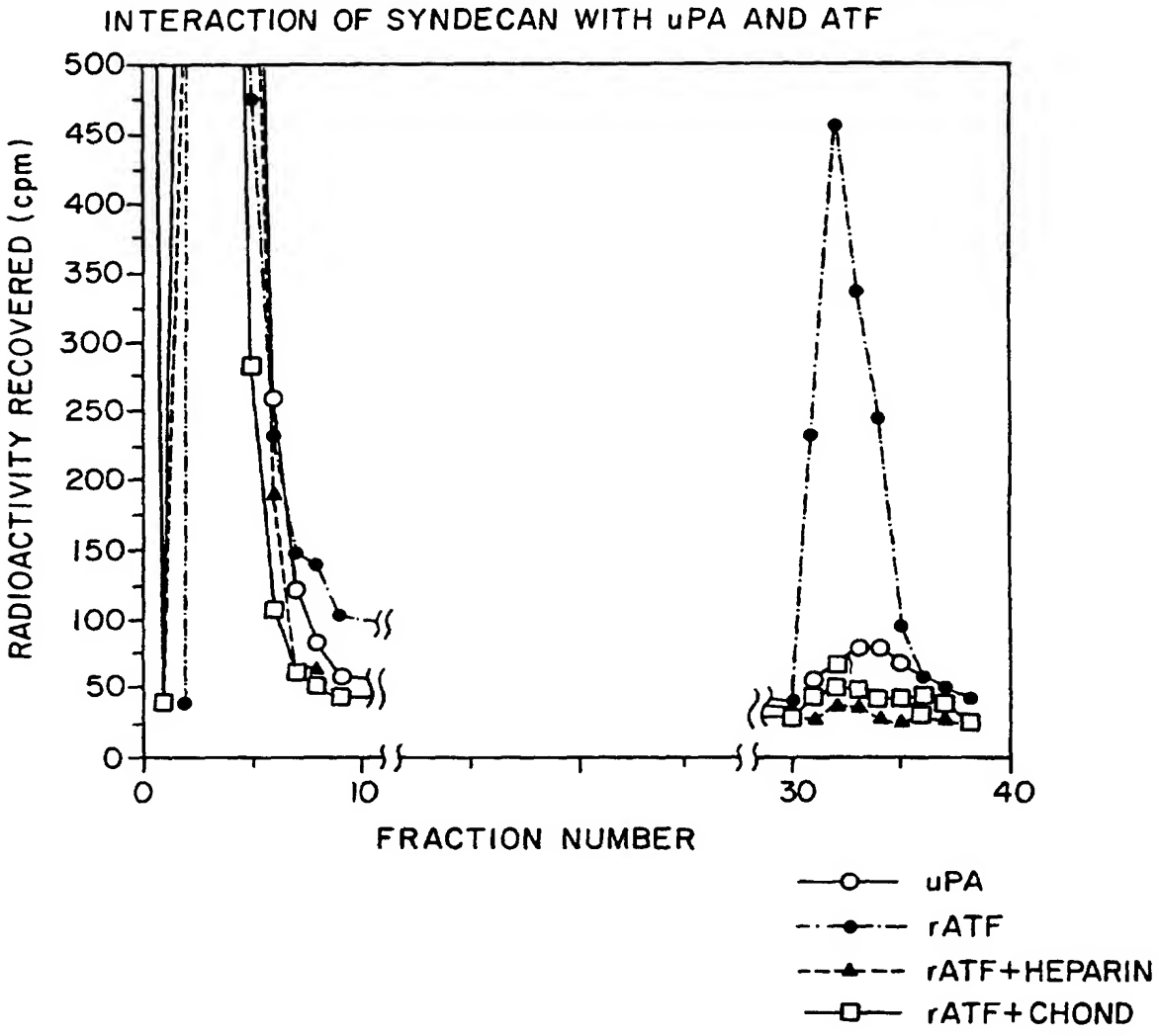

FIG. 13

Mechanism of Growth Stimulation by uPA

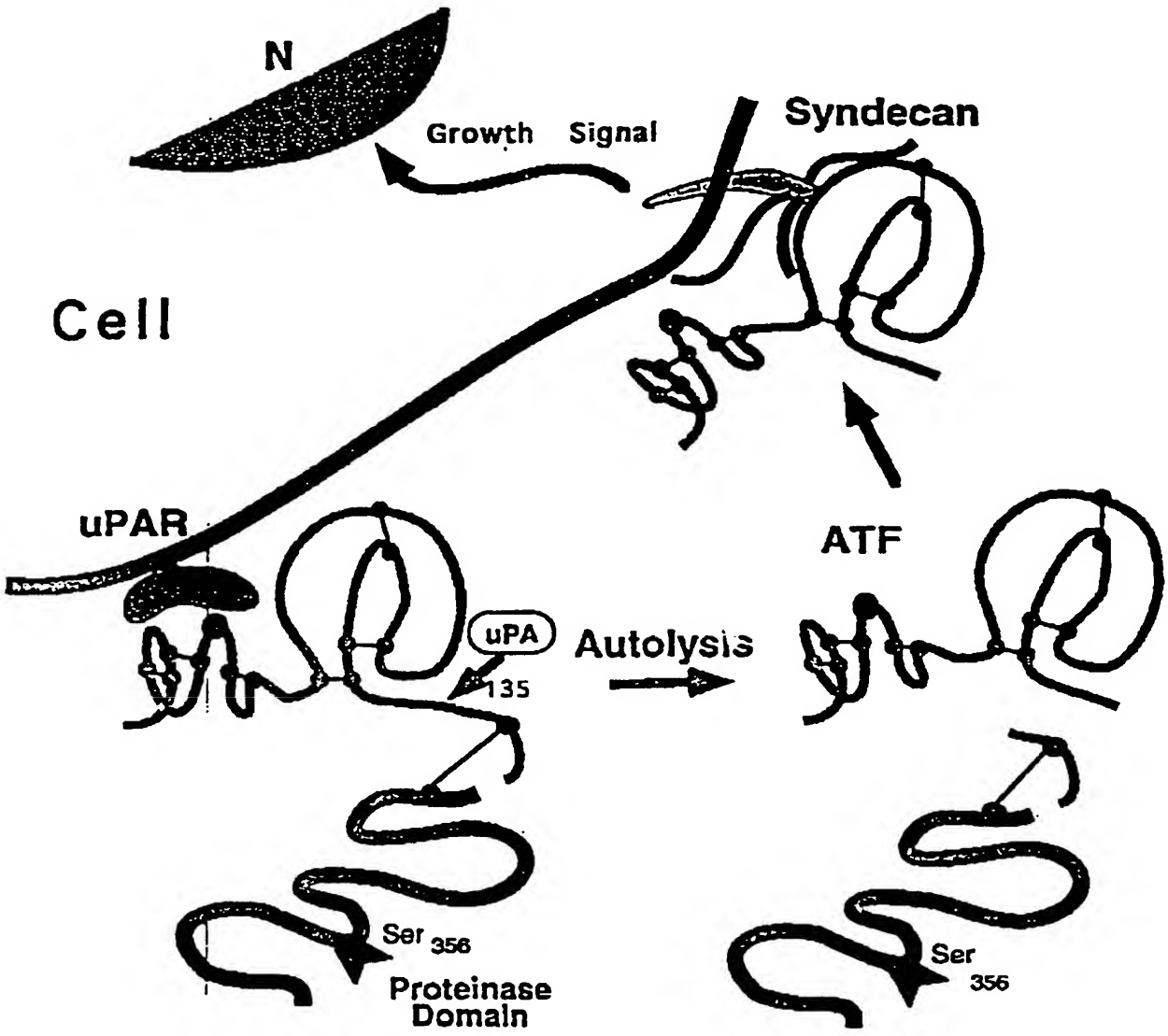

FIGURE 14

INTERNATIONAL SEARCH REPORT

Inter .onal application No.
PCT/US95/12968

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/324, 325, 326, 327, 328, 329, 330; 514/12 13, 14, 15, 16, 17,

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Journal of Biological Chemistry, Volume 267, No 20, issued 15 July 1992, Rabbani et al, " Structural Requirements for the Growth Factor Activity of the Amino-terminal Domain of Urokinase", pp 14151-14156, see entire document.	1-50
A	Journal of Biological Chemistry, Volume 262, No. 10,issued 5 April 1987, Apella et al, " The Receptor-binding Sequence of Urokinase", pp 4437-4440. see entire document.	1-50
Y	Nucleic Acids Research, Volume 13, NO. 8, 20 March 1985, "The Human Urokinase-Plasminogen Activator Gene and Its Promoter, pp 20-33. see entire document. see entire document.	42-50

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 31 JANUARY 1996	Date of mailing of the international search report 13 FEB 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer SG MARSHALL Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

Inter ..onal application No.
PCT/US95/12968

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C07K 7/06, 7/08, 7/10; A61K 38/00

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

530/324, 325, 326, 327, 328, 329, 330; 514/12 13, 14, 15, 16, 17,